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By Major W. D. SUTHERLAND, M.D., I.M.S., *on special duty at the Medical College, Calcutta*, and Captain D. McCAY, M.B., I.M.S., *Professor of Physiology, Medical College, Calcutta.*

From the Physiological Laboratories, Medical College, Calcutta

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We have decided to place on record the following contribution to the action of specific haemolysins and their inhibition, not because the research is by any means complete but because we believe that the results that have been obtained are of considerable interest and of great practical importance, we publish them in the hope that other workers in the same field may be able to follow the side-issues in detail, and that they may be put to the test by practical application.

One of us has already published certain papers (1) giving an account of some experimental work on the haemosozic value of the blood serum; the haemosozic value being understood to indicate those constituents present in the blood serum which preserve the red blood corpuscles from solution in the living body. In the final paper on this subject the significance of a modification of the haemosozic value of the serum by drugs, and the bearing which any lowering of it would have on the etiology of blackwater fever, was discussed.

A certain amount of evidence was brought forward to show that drugs such as mineral acids or acid radicles, which on absorption had a tendency to deplete the plasma or the blood of bases, caused a lowering of the haemosozic value, and therefore diminished the resisting power of the erythrocytes; while drugs whose action on absorption was to increase the number of inorganic ions of the plasma, such as the sodium and calcium chlorides, had the opposite effect, in that they raised the resisting power of the erythrocyte. It was pointed out that if this view were correct, the raising of the haemosozic value of the serum by the administration of chlorides, such as quinine hydrochloride, sodium or calcium chloride, would appear to be a rational indication in the prophylaxis and treatment of the blackwater fever.

The method introduced by Wright (2) for the determination of the haemosozic value was made use of in all these observations, except for the slight but important modification, that the haemolysis obtained with each particular dilution was compared with a standard dilution of one volume of blood in two volumes of distilled water, one in which, therefore, complete haemolysis had taken place. Absolutely comparable results were thus obtained, and the method seemed perfectly satisfactory. However, owing to the criticisms of Christophers and Bentley (3) that Wright's method was not accurate enough to show slight variations in haemolysis produced with different dilutions, a method was devised in which the personal factor was absolutely eliminated.

This brings us to a consideration of the light shed on the problem by the later work we have been able to carry out. These researches we shall take up under two distinct and separate headings:—

A. The evidence afforded by a change in the size of the erythrocytes induced by the administration of sodium and calcium chlorides, acids and drugs. For this section one of us (McCay) alone is responsible.

B. The evidence afforded by the effect of different strengths of saline solution and of calcium chloride on the action of a specific haemolysin. This part of the work has been carried out by both of us in collaboration, but mainly by one of us (Sutherland) who prepared the specific haemolysins.

Part A. The Change in the Size of the Erythrocytes induced by the Administration of Sodium and Calcium Chlorides, Acids and Drugs.

If our contention is correct, that an increase of the inorganic ions present in the plasma accompanies the absorption of salts not foreign to the blood, then, other things being equal, there should be a rise in the osmotic tension of the plasma and a decrease in the size of the erythrocytes by exosmosis. Further, if a decrease in the inorganic ions of the plasma accompanies the absorption of acid salts foreign to the blood, then there should be a fall in the osmotic tension of the plasma and an increase in the size of the erythrocytes from endosmosis. We are not concerned at present with the question as to whether the outer layer of the erythrocyte acts as a semipermeable membrane or not; whatever the explanation may be, we know that a decrease in the number of inorganic ions present in the plasma or suspension fluid is accompanied by an increase in the size of the erythrocyte and vice versa. For example, if, after giving a dose

of sodium chloride and calcium chloride to an animal, we find that the size of the erythrocytes is distinctly decreased, and if, after giving a mineral acid or acid salt, we find the erythrocyte distinctly increased in size, we are justified in assuming that the inorganic ions sodium, calcium, chlorine, are present in excess in the first case while in the second they are diminished, or that some change as suggested by Benjamin Moore, such as adsorption, followed by physical and chemical changes in the haemoglobin, has occurred and is accompanied by an increase in the volume of the red blood corpuscles. Further, in the first case the plasma in which the erythrocytes float will require a greater degree of dilution with distilled water before haemolysis takes place, and in the second case the plasma will require a lesser degree of dilution to produce haemolysis: in other words, the resisting power of the erythrocytes is raised by an increase in the number of inorganic ions present in the plasma and lessened by their decrease. Resistance here means their resistance to haemolysis caused by dilution of the plasma with distilled water.

In the papers already referred to we had put forward evidence to show that the ingestion of chlorides did cause an increase in the resisting power of the erythrocytes, while the ingestion of sulphates had the opposite effect; it therefore remained to prove that with increased resistance of the erythrocyte there was a decrease in its size, and with decrease in its resisting power there was an increase in the size of the erythrocyte.

In order to obtain definite information on this point, after rejecting different methods such as actual measurements of the erythrocytes, percentage volume of erythrocytes and plasma, the following method was devised and the results obtained appear to be fairly free from all inherent sources of error.

From an animal, usually a rabbit, two c.c. of blood was drawn off from a vein. The blood was quickly defibrinated, and a known volume sucked into a large-bore haematocrite tube and the corpuscles centrifugalised in a high-speed electric machine until the volume of corpuscles became constant. This volume was read off and noted.

The clear plasma was now pipetted off and a solution of known composition introduced (N/10 sodium chloride was always used) the corpuscles were shaken up in, and thoroughly mixed with, the saline and allowed to stand for exactly one hour. The emulsion of erythrocytes was again centrifugalised to a constant volume and this volume noted.

The percentage increase in volume due to the substitution of N/10 sodium chloride for the plasma was then calculated. These observations were carried out for two, three or four days for each rabbit, in order to obtain the average increase in the volume of the erythrocytes under normal conditions.

Having obtained this the animal was given the drugs, sodium chloride, calcium chloride, quinine bihydrochloride, quinine sulphate, sulphuric acid, &c., whose effects were under investigation, and exactly the same procedure was followed. In this way any increase or decrease in the volume of the individual corpuscles at once became evident. The necessity of substitution of a known saline solution for the normal plasma was early forced on us by the marked variations met with in the percentage volumes of the plasma and corpuscles when these drugs were given. Thus when an animal was given sodium and calcium chlorides the percentage of corpuscles fell very considerably, the plasma increasing proportionately; at first this was thought to be due to a decrease in the size of the red blood corpuscles, but a little consideration showed it to be really caused by an increase in the percentage of the plasma from a retention of fluid to keep the plasma as nearly isotonic as possible. However, there is a relative as well as an absolute increase in the inorganic ions under the above conditions, the retention of fluid not being able to keep pace with the absorption of ions, so that the osmotic tension of the plasma rises and the size of the erythrocyte falls. The drugs were given to the anaesthetised animals used by means of the stomach tube, so that we knew exactly how much of each particular drug was administered.

Observations were also made on the haemosozic value of the serum during the experiments.

Table I shows the effects of the administration of sodium chloride and calcium chloride on the erythrocytes and plasma.

This investigation shows that, in a rabbit, the administration of chlorides is followed by:—

1. A decrease in the salt content equivalent of the serum, i.e., the corpuscles are able to stand a greater and greater dilution of a normal salt solution before haemolysis takes place, showing an increased resisting power. In the table we find that haemolysis normally occurs when one volume of blood is mixed with two volumes of N/45 sodium chloride, but after the administration of the third dose of chlorides haemolysis only took place when N/70 sodium chloride was substituted for the N/45 sodium chloride. As a sequela of this we also find the haemosozic value of the serum much increased after the administration of chlorides.

TABLE I

NORMAL

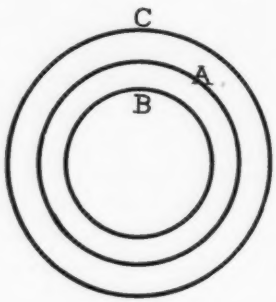
Date	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Normal percentage increase due to 0·1 N sodium chloride	
27.1.08	0·130	0·94	41·45	47·06	13·29	This shows an average increase in volume of the red corpuscles when N/10 sodium chloride is substituted for the plasma of 12·28 per cent.
28.1.08	0·137	0·98	46·40	51·42	10·81	
11.2.08	—	—	45·06	50·56	12·26	
12.2.08	0·130	1·04	47·80	53·90	12·76	

AFTER DRUGS

Date	Drugs	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Percentage increase due to 0·1 N sodium chloride	Percentage decrease due to the increased salinity of serum from drugs	
28.1.08	Cal. Chlor. grains v Sodium Chlor. grains xxx	0·123	1·23	37·63	?	?	?	2 hours after first dose was given.
29.1.08	Nil.	0·123	0·984	39·57	46·28	16·95	4·67	24 hours after first dose of drugs.
29.1.08	Dose (above)	0·097	1·16	35·42	41·98	18·52	6·24	2 hours after second dose of drugs.
30.1.08	Nil.	0·116	1·16	33·40	39·75	19·00	6·72	24 hours after second dose of drugs.
30.1.08	Dose (above)	0·097	1·63	26·80	33·33	24·37	12·09	2 hours after third dose of drugs.
31.1.08	Nil	0·083	1·83	22·80	28·42	24·65	12·37	24 hours after third dose of drugs.
1.2.08	Nil	0·093	1·12	31·61	37·38	17·93	5·65	48 hours after last dose of drugs.
3.2.08	Nil	0·1016	1·16	30·26	35·52	17·38	5·10	72 hours after last dose.
4.2.08	Nil	0·116	0·95	32·90	37·03	12·55	0·27	96 hours after last dose.
6.2.08	Nil	0·123	0·97	38·56	43·71	13·35	1·07	144 hours after last dose.
11.2.08	Nil	—	—	45·06	50·56	12·26	Nil	11 days after last dose.
—	Nil	0·130	1·04	47·80	53·90	12·76	0·48	12 days after last dose.

The effects of the drugs are well marked up to from 48-72 hours after stopping their use.

2. The next point of importance brought out by the investigation is the very great decrease in the percentage volume of the corpuscles with a corresponding increase in that of the plasma. Thus in the rabbit normally the relations of corpuscular elements to plasma are 45 and 55 per cent. As soon as the chloride treatment was begun a fall in the percentage volume of the corpuscles took place, and after the third dose (in all calcium chloride 15 grains and sodium chloride 90 grains had been ingested) the volume of the corpuscles had fallen to 23 per cent., and that of the plasma had risen to 77 per cent. This can only be explained by retention of water in the attempt to maintain the isotonicity of the plasma, and, perhaps to some extent, by more corpuscles being removed during defibrination—the formation of fibrin having been increased by the increased calcium content of the plasma.



3. Under the conditions of the experiment we found that the average increase in volume of the corpuscular elements when N/10 sodium chloride was substituted for the plasma was normally 12·28 per cent. Reference to the table will show that we obtained a very much greater increase than this after chlorides had been administered. Thus twenty-four hours after the third dose the increase in the volume of the corpuscles, on substituting N/10 sodium chloride for the plasma, reached the high figure of 24·65 per cent. The explanation of this is fairly obvious. Suppose in the accompanying diagram that the circle A represents the size of the normal erythrocyte in its own plasma, substitution of N/10 sodium chloride for this plasma causes a 12 per cent. increase in the volume of the corpuscle, which we shall say is represented by the circle C. The rabbit is then given large doses of sodium chloride and calcium chloride, and it is found that substitution of N/10 sodium chloride for the plasma now produces a 24 per cent. increase in the

volume of the corpuscle: therefore, since C represents the volume in N/10 sodium chloride and A represents a 12 per cent. reduction in the volume, the circle B will represent a 24 per cent. reduction, that is, we have practical proof that the administration of chlorides does cause a decrease in the size of the red blood corpuscle, and that this smaller decrease in the size of the red blood corpuscle, and, as we have already shown, this smaller corpuscle is much more difficult to break up by the dilution method than the normal sized corpuscle.

We may therefore conclude that the results obtained by this method afford confirmatory evidence of the increase in the resisting power of the erythrocyte caused by the ingestion and absorption of chlorides, and that the administration of sodium and calcium chloride is accompanied by an increase in the relative number of the inorganic ions present in the plasma.

Another series of observations was made in which 20 grains of sodium chloride and 4 grains of calcium chloride were given daily to a rabbit; the results, while not so marked, undoubtedly pointed to the same conclusion, for the same increased resistance of the corpuscles to a dilution of the plasma, and the same reduction in the volume of the erythrocytes followed the ingestion of these drugs.

Further proof that the administration of calcium chloride is accompanied by an increase in the calcium content of the blood is afforded by the fine piece of research on this subject by Boggs (4). This observer experimenting on dogs has proved that calcium given by the mouth does cause a great increase—up to 36 per cent.—of the calcium content of the blood, and that this increase is maintained for several days after cessation of the administration of the salt.

The experiments of Grübe on the alteration of the molecular concentration brought about by the ingestion of hypotonic and hypertonic mineral waters would appear to put it beyond all doubt that the osmotic tension of the blood can be very considerably modified: a decrease following the ingestion of hypotonic and an increase the ingestion of hypertonic mineral waters (5).

As we go to press further confirmation of the above views comes in an article by Mendel and Benedict (6), who conclude that a considerable quantity of the excess of calcium introduced (intravenously) may be retained for some time in the body.

Another point of importance in connection with the therapeutic use of calcium in blackwater fever is its diuretic effect when given intravenously with sodium chloride solution, suppression of urine being often a marked sign in severe cases of blackwater fever.

Table II

NORMAL

Date	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Normal percentage increase due to 0·1 N sodium chloride	
11.2.08	0·135	0·98	45·06	50·56	12·26	From these and other observations the normal percentage increase in the volume of the corpuscles on substitution of N/10 sodium chloride for the plasma = 12·28 per cent.
12.2.08	0·130	1·04	47·80	53·90	12·76	

AFTER DRUGS

Date	Drugs	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Percentage increase due to 0·1 N sodium chloride	Percentage decrease due to the increased salinity of serum from drugs	
13.2.08	Cal. Chlor. grains iv Sod. Chlor. grains xxii	0·1016	1·17	46·34	52·44	13·16	0·88	24 hours after first dose.
14.2.08	,,	0·0974	1·168	41·96	49·14	17·11	4·83	24 hours after second dose.
15.2.08	,,	0·0974	1·18	41·92	49·94	19·84	7·56	24 hours after third dose.
16.2.08	,,	0·087	1·25	41·18	50·00	21·40	9·12	24 hours after fourth dose.
17.2.08	,,	0·0873	1·09	39·63	48·50	22·13	9·85	24 hours after 5th dose.
18·2·08	Nil	0·085	1·24	35·82	42·87	19·68	7·40	48 hours after 5th dose.
19.2.08	Nil	—	—	—	—	—	—	—
20.2.08	Nil	0·1016	1·176	41·44	47·36	14·28	2·00	96 hours after fifth dose.

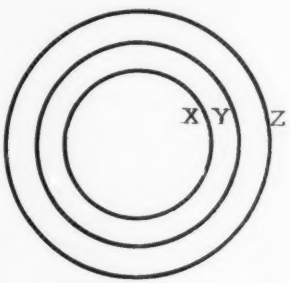
Working by the same method, after the administration of acid sulphates and sulphuric acid we got distinct evidence that these drugs have an effect directly opposed to that of chlorides on the volume and resisting power of the erythrocytes. Owing, however, to the pressure of other duties, these observations had to be stopped before they had reached completion. Table III gives some of the typical results obtained.

Comparison of the results of Table III with those of Tables I and II shows very distinct differences under the three headings:—

1. There is no decrease, but an increase, in the salt content equivalent of the blood, so that the erythrocytes are unable to withstand identical dilutions of the plasma with distilled water before and after the administration of sulphates.

2. There is no fall in the percentage volume of the corpuscles, but on the contrary a tendency to increase—a very marked difference from what was found to be the case with chlorides.

3. When N/10 sodium chloride is substituted for the plasma before and after the administration of sulphates, the increase in the volume of the corpuscles, instead of showing the normal average for healthy erythrocytes, is considerably less than the normal, and this again contrasts very markedly with the effects of chlorides. The following figure will make this clear:—



Suppose the circle X represents the volume of the normal corpuscle before any drugs are given, and Z represents it when the plasma is replaced by N/10 sodium chloride. Now we find that after sulphates have been given the increase in volume of the corpuscles is not nearly so great on substituting N/10 sodium chloride for the plasma as when no sulphates have been given; therefore the size of the corpuscle will lie between normal and its size in N/10 sodium solution, i.e. at Y.

TABLE III

THE ACTION OF SULPHATE ON THE ERYTHROCYTES

NORMAL

Date	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Normal percentage increase due to 0·1 N sodium chloride	
20.1.08	0·130	0·92	42·50	46·25	8·82	This gives an average increase in the volume of the corpuscles when N/10 sodium chloride is substituted for the plasma of 10·47 per cent.
21.1.08	0·135	0·912	36·04	40·10	11·26	
21.1.08	0·135	0·85	40·77	44·07	11·00	
25.1.08	0·116	1·16	34·57	38·30	10·79	

AFTER DRUGS

Date	Drugs	Percentage salt content equivalent	Percentage haemosozic value of serum	Percentage volume of corpuscles	Percentage volume of corpuscles in 0·1 N sodium chloride	Percentage increase due to 0·1 N sodium chloride	Percentage increase due to the decreased salinity of serum from drugs
21.1.08	Quin. sulph. grains ii Mag. sulph. grains. ii s.s. Ac. sulph. dil. min. ii s.s.	0·135	0·85	40·77	44·84	10·98	0·51
22.1.08		0·146	1·02	38·35	39·93	4·12	6·25
22.1.08		0·123	0·921	40·83	42·75	4·70	5·77
23.1.08		0·146	1·02	40·59	—	—	—
24.1.08		0·130	0·87	39·52	39·71	0·49	9·98
25.1.08		0·116	0·87	42·76	43·38	1·45	9·02

We are justified from these observations in concluding that the administration of sodium, calcium, chlorine, etc., has a marked influence in increasing the resisting power of the erythrocyte to haemolysis by dilution, while at the same time causing a very distinct decrease in its size; and that the administration of sulphates and mineral acids has the opposite effect, though not to such a marked extent.

As it appeared to be likely that in blackwater fever there is something present in the blood that tends to lower the resisting power of the erythrocytes, it was reasoned that in this disease sulphates and mineral acids should not be given, but that the administration of chlorides and inorganic basic salts is indicated, in the hope of causing thereby an increase of this resisting power. It was considered that even if this 'something' be a specific haemolysin,[1] the chlorides would have a good effect on cases which are, so to speak, on the brink, and likely to be precipitated into the abyss if their equilibrium be upset: if the cells are just able to withstand the amount of haemolysin present, on the ingestion of chlorides their power of resistance would be increased, whereas the ingestion of sulphates or mineral acids would cause them to succumb. Again, supposing that the patient were suffering from an attack of blackwater fever, the ingestion of sulphates or mineral acids would tend to turn the scale in favour of the haemolysin and against the resistant powers of the organism, and so would hasten the fatal issue. It was not suggested at the time that the specific action of the haemolysin would be interfered with by an increase in the amount of inorganic ions present in the blood.

After a year and a half the opportunity came of testing the effect of different strengths of solutions of saline and of calcium chloride on the action of specific haemolysins.

In the meantime the valuable research on blackwater fever by Christophers and Bentley appeared, in which these observers also favour the view of the presence of an haemolysin as the causative agent.

They made two most important observations, which are decidedly confirmatory of the views we have expressed, and which in turn our work on specific haemolysins recorded below has corroborated. These are:—

1. 'In comparing the drawings of blood films it became evident in several instances that in a series of films taken at short intervals within

1. That a specific haemolysin is the cause of blackwater fever is rendered very probable by the fact that in paroxysmal haemoglobinuria a specific haemolytic amboceptor has been demonstrated by various observers, including Meyer and Emmerich (*Deut. Archiv. f. klin. Med.*, June, 1909.)

the first twenty-four hours of the onset of blackwater fever, all the elements of the blood, both red cells and leucocytes, were on a larger scale in those taken later in the attack than in the earlier ones.' (7) This is confirmatory of our view that the resisting power of the corpuscle being decreased either by the action of a specific haemolysin, or by depleting the plasma of inorganic ions, an increase in the size of the corpuscle will take place.

2. They found that there was a marked raising of the isotonic point of the erythrocytes after the injection of haemolytic sera or snake-venom into animals (8). However, they say they have not found any marked raising of the isotonic point in blackwater fever, although the figures in the three cases seen early in the disease hardly bear out this contention (9).

They consider a specific haemolysin to be the most probable cause of blackwater fever, and we shall be able to produce strong evidence of the inhibitory influence of saline hypertonic solutions and of calcium salts on the action of haemolysins. This in turn will again confirm the view which we have expressed as to the superiority of the chloride salts of quinine over the sulphate, and of the value of therapy by which inorganic ions are introduced into the plasma in the prophylaxis and treatment of blackwater fever.

B. The Inhibitory Effect of Hypertonic Saline Solutions and Calcium Salts on the Action of a Specific Haemolysin

That a specific haemolysin *sui generis* is present in blackwater fever may justifiably be assumed from the results of Myers and Emmerich's work on paroxysmal haemoglobinuria quoted above. As we had no case of blackwater fever before us, we have not been able to demonstrate the presence of this haemolysin. But we have been able to carry out a considerable amount of work with a haemolysin for the erythrocytes of the sheep, and we believe that the results obtained by us *in vitro* are valuable indications of what may be expected to occur *in vivo*.

At first we examined the question whether any modification of the action of a haemolytic system occurs when the saline solution used varies from that usually employed. For reasons which will at once occur to our readers we preferred a definite haemolytic system to a normal serum that had a haemolytic action on sheep's erythrocytes. We believe that we have established beyond doubt that the results of

the action of the haemolytic system on the erythrocytes vary inversely with the degree of salinity of the medium, and that the presence of excess of calcium salts has a most marked inhibitory effect on this action.

The inhibitory influence of calcium and hypertonic saline solutions on haemolysis we assume to be due to an increase of the resisting power of the erythrocytes, on the ground of the observations detailed above. We have not been able to obtain evidence that the haemolysin itself is affected; but even assuming that it is because the haemolysin is affected that its specific action is inhibited, the *therapeutic indication* to be drawn from our observations remains the same.

In the following experiments we employed haemolytic antisera obtained from rabbits, fowls, and a goose that had been immunized by means of injections of washed sheep's erythrocytes. The erythrocytes were washed three times for the injections, and for making the indicator, as we found that this amount of washing was enough to deprive them of all serum. Standardization of the power of the haemolytic antiserum was carried out in the usual way, the antiserum being heated to 56° C. for half an hour to render it inactive, and then reactivated at the time of experiment by the addition of fresh guinea-pig serum. We have found that 0·08-0·1 c.c. of fresh guinea-pig serum is enough, and that this will not of itself cause any haemolysis of the indicator, which was in every case a 5 per cent. suspension of thrice-washed sheep's erythrocytes, the medium in which these were suspended being indicated in the case of each experiment. For standardization, of course, a 0·85 per cent. sodium chloride solution was used.

Experiment I.—Twice the minimum haemolytic dose of amboceptor A (anti-sheep erythrocyte, rabbit's serum), whose minimal haemolytic dose was 0·0035 c.c., was used in each tube, as complement 0·08 c.c. of fresh guinea-pig's serum, and the total volume made up to 1 c.c. with the various concentrations of sodium chloride salines shown in the table as follows:—

Indicator—1 c.c. 5 per cent. suspension in 0·85 per cent. sodium chloride.

1.	N/2	No haemolysis
2.	N/3	Haemolysis slightly inhibited
3.	N/4	Haemolysis complete
4.	N/5	,,
5.	N/6	,,
6.	0·85 per cent.	,,
7.	N/8	,,
8.	N/9	,,

Experiment II.—Amboceptor 0·0035 c.c. = 0·7 c.c. of 1 : 200 dilution. Other conditions as in Experiment I.

1.	N/3	No haemolysis
2.	N/4	Haemolysis slightly inhibited
3.	N/5	Haemolysis complete
4.	N/6	,,
5.	0·85 per cent.	,,
6.	N/8	,,
7.	N/9	,,
8.	N/10	,,

Experiment III.—Haemolytic amboceptor B minimal haemolytic dose = 0·0075 c.c. = 0·75 c.c. of 1 : 100 dilution. Complement : 0·1 c.c. guinea-pig's serum in each tube.

Indicator 5 per cent. suspension washed sheep's erythrocytes in the various strengths of salines used. The dilution of amboceptor and the making up of the quantities of this to 1 c.c. were carried out with 0·85 per cent. sodium chloride.

Amboceptor c.c.	N/4	N/5	N/6	0·85 per cent.	N/10
0·01	Inhibition fair	Trace of inhibition	Haemolysis complete	Haemolysis complete	Haemolysis complete
0·0075	Inhibition greater	Inhibition greater	Some inhibition	Haemolysis complete	Haemolysis complete
0·005	Still greater inhibition	Inhibition greater	Greater inhibition	Some inhibition	Nearly complete haemolysis
0·0025	Scarcely any haemolysis	Inhibition still greater	Greater inhibition	More inhibition	Some inhibition
0·0015	Trace of haemolysis	Inhibition still greater	Slight haemolysis	More inhibition	More inhibition
0	No haemolysis	No haemolysis	No haemolysis	No haemolysis	Slight haemolysis

Thus we have slight haemolysis caused by the complement alone in the last tube of the N/10 series, in which series all the tubes show more haemolysis than the corresponding tubes of other series. We note too, that N/6 (0·98 per cent. sodium chloride) inhibits the action of 0·0075 c.c. amboceptor, which quantity caused complete haemolysis in the 0·85 per cent. and N/10 series.

Experiment IV.—Amboceptor C minimal haemolytic dose = 0·01 c.c. = 1 c.c. of 1 : 100 dilution. Complement : guinea-pig's serum 0·1 c.c.

Indicator 5 per cent. suspension of washed sheep's erythrocytes in various strengths of sodium chloride solution shown.

The dilutions of amboceptor and the making up of the quantities of this to 1 c.c. in each tube were carried out with the various strengths of sodium chloride solution noted at the head of each column.

Amboceptor c.c.	N/4	N/5	N/6	0·85 per cent.	N/10
0·01	No haemolysis	About three-quarters haemolysed	Nearly complete haemolysis	Complete haemolysis	Complete haemolysis
0·0075	No haemolysis	About half haemolysed	About three-quarters haemolysed	Nearly complete haemolysis	Complete haemolysis
0·005	No haemolysis	Trace of haemolysis	About half haemolysed	About three-quarters haemolysed	Complete haemolysis
0·0025	No haemolysis	No haemolysis	Some haemolysis	Some haemolysis	About three-quarters haemolysed
0·0015	No haemolysis	No haemolysis	Some haemolysis	Trace of haemolysis	About half haemolysed
0	No haemolysis	No haemolysis	No haemolysis	No haemolysis	Some haemolysis

From this it is evident that any solution of sodium chloride that contains more than 0·85 per cent. of this salt is able to inhibit haemolysis in proportion to the percentage of salt that it contains, while any solution that contains less than 0·85 per cent. permits haemolysis to take place with ease proportionate to the lessening of its salt content.

The haemolytic action of a specific haemolysin varies then inversely with the strength of the saline solution in which the corpuscles of the indicator float. Thus the corpuscles' resisting power is so increased by strong saline that a specific antiserum which can cause complete haemolysis of corpuscles in 0·85 per cent. has little or no effect when the corpuscles are suspended in a 1·46 per cent. solution, and only half its effect when they are suspended in a 1·17 per cent. solution.

Experiment V.—In this experiment we endeavoured to find out what minimum amount of calcium chloride would inhibit the action of the specific haemolytic amboceptor.

Amboceptor C minimal haemolytic dose 0·01 c.c. = 0·1 c.c. of 1 : 10 dilution in each tube.

Complement 0·1 c.c. of fresh guinea-pig's serum.

Indicator 1 c.c. of 5 per cent. suspension of washed sheep's erythrocytes in 0·85 per cent. sodium chloride solution.

Dilutions of calcium chloride, of which the required quantities were taken, and where necessary made up to 1 c.c. with 0·85 per cent. sodium chloride.

Tube	Strength of calcium solution used	Amount of calcium solution added c.c.	Result	Contents of tube 2·2 c.c. Amount of calcium chloride present
1	1 : 100 in 0·85 per cent. sodium chloride	1·0	Very little haemolysis	0·01 grm.
2	[Quantities made up to 1 c.c. with 0·85 per cent.]	0·75	A little more haemolysis	0·0075
3	—	0·5	Still more haemolysis	0·005
4	—	0·25	Still more haemolysis	0·0025
5	—	0·15	About half haemolysed	0·0015
6	1 : 1000	1·0	More haemolysed	0·001
7	—	0·75	Still more haemolysis	0·00075
8	—	0·5	Still more haemolysis	0·0005
9	—	0·25	Still more haemolysis	0·00025
10	—	0·15	About three-quarters haemolysed	0·00015
11	—	0	Complete haemolysis	0
12	No amboceptor in this tube	0	Practically no haemolysis	0

From this the inhibitory power of calcium chloride *per se* is evident, for even so small a quantity as 0·0015 gm. of this salt in 2·2 c.c. of tube contents lessened the haemolytic power of the amboceptor by half, and the tenth of this quantity had a distinct inhibitory effect.

Experiment VI.—Haemolytic amboceptor of which minimal haemolytic dose as yet undetermined. A 1 : 100 dilution of this with various solutions was used in varying quantities, and these were made up to 1 c.c. with the various solutions, where necessary.

In each tube 0·0015 grm. of calcium chloride, save where indicated.

Complement = 0·1 c.c. fresh guinea pig's serum.

Indicator 1 c.c. of 5 per cent. suspension of washed erythrocytes in the various solutions.

Amboceptor dilution 1 : 100	N/5	N/6	0·85 per cent.	N/10
1·0 c.c.	Complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
0·75 „	Two-thirds complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
0·5 „	Half complete haemolysis	Three-quarters complete haemolysis	Complete haemolysis	Complete haemolysis
0·25 „	Quarter complete haemolysis	Half complete haemolysis	Complete haemolysis	Complete haemolysis
No calcium chloride and no amboceptor	No haemolysis	No haemolysis	No haemolysis	Trace of haemolysis

On standardising the amboceptor used in this case, it was found that its minimal haemolytic dose was 0·001 c.c. = 0·1 c.c. of 1 : 100 dilution. Notwithstanding the fact that much more than this had been used in the experiment, the inhibitory power of hypertonic salt solution containing calcium chloride is very evident, and is confirmed by the following experiment :—

Experiment VII.—Haemolytic amboceptor D minimal haemolytic dose = 0·0004 c.c. = 0·4 c.c. of 1 : 1000 dilution with various solutions.

Complement : 0·1 c.c. guinea-pig's serum.

In each tube, save where noted, was 0·0015 grm. of calcium chloride.

Indicator 5 per cent. suspension of erythrocytes in the various strengths of saline solution.

Quantities of amboceptor made up where necessary to 1 c.c. by addition of the required solution.

Amboceptor	Calcium chloride 0·0015 grm. in each tube				No calcium chloride
1 : 1000 dilution	N/5	N/6	N/10	0·85 per cent.	0·85 per cent.
0·8 c.c.	Half complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
0·6 „	Half complete haemolysis	Half complete haemolysis	Complete haemolysis	Three-quarters complete haemolysis	Complete haemolysis
0·4 „	Quarter complete haemolysis	Half complete haemolysis	Complete haemolysis	Half complete haemolysis	Complete haemolysis
0·2 „	Practically no haemolysis	Trace of haemolysis	Nearly complete haemolysis	Trace of haemolysis	Three-quarters complete haemolysis
0·1 „	No haemolysis	No haemolysis	Less complete haemolysis	Slight trace of haemolysis	Half complete haemolysis
0	No haemolysis	No haemolysis	Trace of haemolysis	No haemolysis	No haemolysis

The following experiment gives a further control for the above results.

Experiment VIII.—Amboceptor not standardised diluted 1 : 1000 with various solutions.
Complement: 0·1 c.c. guinea-pig's serum.
Control 1 c.c. of 5 per cent. suspension of erythrocytes in the various solutions.
Quantities made up to 1 c.c. where necessary with various dilutions.
Series A. Calcium chloride 0·0015 grm. in each tube. Series B. No calcium chloride.

	Amboceptor 1 : 1000 dilution	N/5	N/6	N/10	0·85 per cent.
A	0·8 c.c.	Complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
	0·6 „	No haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
	0·4 „	No haemolysis	Partial haemolysis	Complete haemolysis	Partial haemolysis
	0·2 „	No haemolysis	No haemolysis	Partial haemolysis	No haemolysis
	0·1 „	No haemolysis	No haemolysis	No haemolysis	No haemolysis
B	0·8 „	Complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
	0·6 „	Complete haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
	0·4 „	Partial haemolysis	Complete haemolysis	Complete haemolysis	Complete haemolysis
	0·2 „	No haemolysis	Partial haemolysis	Complete haemolysis	Complete haemolysis
	0·1 „	No haemolysis	No haemolysis	Complete haemolysis	Complete haemolysis
	0	No haemolysis	No haemolysis	No haemolysis	No haemolysis

Amboceptor standardised and minimal haemolytic dose found to be 0·00008 c.c. = 0·08 c.c. of 1 : 1000.

Having thus established the inhibitory effect of hypertonic saline solutions and calcium on the action of the haemolytic system, we attempted to discover whether this inhibition was due to any modification of the haemolytic system. To this end we carried out the following experiment.

In series A the tubes contained amboceptor which had been acted upon by the N/5 solution for an hour; in series B the corpuscle-suspension used as indicator had been prepared with N/5 solution an hour before the experiment was carried out; and in series C the guinea-pig serum had been for an hour subjected to the action of N/5 solution, being otherwise under the same conditions as that used for the other series.

The control is furnished by the tubes of series D, in which the haemolytic amboceptor's standardization was, so to speak, confirmed. This amboceptor had been obtained from a goose, and was, as is evident, of but slight potency.

We may note that so far we have had only small success in the immunization of geese, whereas fowls have yielded excellent antisera of very high potency, when immunised with cell-free serum or washed erythrocytes.

Experiment IX.—

Amboceptor	A N/5	B N/5	C N/5	D 0·85 per cent.
0·8 c.c.	No haemolysis	No haemolysis	No haemolysis	Complete haemolysis
0·6 „	No haemolysis	No haemolysis	No haemolysis	Half complete haemolysis
0·4 „	No haemolysis	No haemolysis	No haemolysis	Quarter complete haemolysis
0·2 „	No haemolysis	No haemolysis	No haemolysis	Trace of haemolysis
0·1 „	No haemolysis	No haemolysis	No haemolysis	No haemolysis

0·1 c.c. guinea-pig's serum as complement.

1 c.c. 5 per cent. suspension of erythrocytes in N/5 for A, B, and C, and in 0·85 per cent. sodium chloride for D, as indicator. Control tube for complement. No amboceptor. No haemolysis.

Obviously no conclusion can be drawn from this experiment, save that it confirms the results of the others as to the inhibitory power of N/5. Nor did repetition of the experiment, carried out by us with the very greatest care, shed any further light on the subject, although the progress of haemolysis in the tubes was noted every fifteen minutes.

Suggestions as to the Therapy of Blackwater Fever

On the basis of the results obtained from the administration of sodium and calcium chlorides in increasing the resisting power of the erythrocyte and of diminishing its size, also from the proved influence of hypertonic saline solutions and of calcium in inhibiting the action of specific haemolysins, the natural deductions are obvious.

Christophers and Bentley have shown that in blackwater fever the corpuscles, both red and white, are larger than normal and, therefore, according to our view, more vulnerable.

They have also proved that the action of specific haemolysins is

accompanied by a raising of the isotonic points,[1] i.e., the red blood corpuscles instead of being able to resist dilutions of the plasma down to what corresponds to 0·47 per cent. saline, as they can normally, are dissolved in solutions of 0·6, 0·7, 0·8, and even in 0·9 per cent. in some cases. Their negative finding of any raising of the isotonic point in the cases of blackwater fever investigated may be explained by the presence of an anti-haemolysin, or by the difficulty of knowing when haemolysis due to the saline begins to take place in a condition where haemoglobinaemia is already present. But we cannot accept their results, shown on page 130, Scientific Memoirs, No. 35, as proving their contention that there is no raising of the isotonic point. In every case they examined on the day of onset there was haemolysis with N/12 sodium chloride, and even with lower dilutions—the average for normal individuals being N/14 or N/15 sodium chloride. This fact is well brought out in the records of the several cases [XX, XXVI and XXVII] where comparisons with controls were carried out. In every case of haemoglobinuria the blood was more easily broken down than the normal blood by dilutions of saline. We think that further work will bring the condition of the isotonic point in blackwater fever into line with the acknowledged rise that takes places in other conditions which are brought about by the action of a specific haemolysin. It is not stated whether death took place in any of these recorded cases; of course it is to be expected that in recovery there would be no rise of the isotonic point but that, on the contrary, this would fall. Further, in most of the cases in which they determined the isotonic point the case was 'mild,' or the observations were made some days after the onset of the disease—it may well be that the condition of affairs in these circumstances is by no means that which obtains just before, or at, the onset of the attack. Besides it is very possible that the presence of anti-haemolysins in all the cases they had under observation, might go far to obscure the results.

However, leaving all this aside, the facts that the corpuscles are enlarged, and that the isotonic point is disturbed in blackwater fever, afford strong evidence that the condition of the erythrocytes is one of increased vulnerability. To whatever this may be due, we have shown that it is possible by increasing the inorganic ions in the plasma to prevent

1. We do not agree with the meaning that these observers seem to attach to the term 'isotonic point.' The haemolytic point would appear to be the correct term, or, as might be said in German, the 'Haemolyse-Anfangspunkt.'

haemolysis in conditions where otherwise haemolysis would have taken place, and this even in the case of a specific haemolysin.

The suggestions we make with regard to the treatment of blackwater fever are:—

That any drug having a tendency to decrease the number of inorganic ions present in the plasma be eschewed. Such drugs as sulphates, acid salts, mineral acids, and even magnesium sulphate—unless given in doses sufficient to cause purgation and as a consequence to prevent its absorption—would come into this category.

That the administration of drugs that tend to increase the number of inorganic ions present in the plasma is indicated.

Such drugs are sodium chloride or carbonate, neutral salts of vegetable acids, calcium chloride, lactate or carbonate, or, if necessary, quinine bihydrochloride, hydrobromide or tannate.

In all cases where blood destruction is at all marked and where the patient is seriously ill, medication by the mouth will probably be of little avail. In such cases we are firmly of the opinion that *intravenous injection* of hypertonic saline to which a little calcium has been added, or of Ringer's fluid to which extra sodium chloride has been added, will be of the greatest service. In combination with the intravenous injection, medication by the mouth may be tried if vomiting is absent, but it is very doubtful indeed if any adequate absorption from the intestinal tract can take place in patients suffering severely from a condition like blackwater fever; and we know, from Dixon's (10) work, that it is only when it is subcutaneously administered that calcium chloride can be expected to cause a rise of the calcium content of blood, for when given by the mouth the salt is eliminated *pari passu* with its absorption.

The solution we recommend is this:—

Sodium chloride	1·2 grammes.
Calcium chloride	0·025 grammes.
Aquae destill	100 c.c.

For ordinary work, where it would be impossible to have these quantities accurately determined in time, two tea-spoonsful of common salt in a pint of boiled water can be made use of; if available three grains of calcium chloride should be added, or injected hypodermically, in saline solution.

The amount to be given should not be less than one pint, and may

be determined by the condition of the patient. Repeated injections of small amounts would probably be found to give the best results.

We have shown that the smallest trace of calcium has a marked effect, and that the raising of the salinity of the blood plasma to 1 per cent. will absolutely inhibit haemolysis due to specific haemolysins, even when these are present in sufficient amount to cause complete haemolysis of a known suspension of corpuscles.

Now in blackwater fever there is never, even in the most virulent case, a sufficient quantity of the haemolysin present to cause complete haemolysis of all the erythrocytes in the body; so that our task of preventing haemolysis should be much easier than in the experiments above detailed. It would not therefore be necessary to raise the salinity of the plasma to anything like 1 per cent. in order to inhibit the action of the active agent in blackwater fever. For this reason we recommend that treatment should be carried out by beginning with one pint of 1·2 per cent. saline intravenously, the physician being guided by the condition of the patient as to the necessity for repeating the injection.

Corroborative evidence of the accuracy of these views is afforded by the success of the Hearsey method of treatment, which is the only method that can really claim comparative success.

This consists in giving sodium bicarbonate and perchloride of mercury, and is, after all, only another method of increasing the inorganic salts of the plasma—the end aimed at in our treatment. It is remarkable and well worthy of note that Dr. Hearsey forbids all *acid* drinks and lays stress on the *antacid* action of his combination. This method would fail, of course, in the very conditions in which treatment is most necessary, viz., in those cases which are so severe that absorption from the alimentary canal does not take place, and in those in which vomiting is present and persistent.

Acting on the views herein detailed Dr. R. L. Patterson, of Assam, reports several successful results with calcium chloride, and one very suggestive case treated with intravenous injection of hypertonic saline solution.[1] He writes that the patient was dying, 'I at once opened a vein and transferred several pints of hypertonic saline. There was a temporary rally, and the urine drawn off with a catheter a few hours later was clear, but the patient died the same night. I hope to catch my next case early, and transfuse as once.'

1. Dr. Patterson is the first, to our knowledge, who has treated cases of blackwater fever in this way.

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2. *The Indian Medical Gazette*, 1907, 1908.

3. *The Bio-Chemical Journal*, Vol. III, No. 3, 1908. See also Wright and Kilner, *Lancet*, 1904. Wright and Ross, *Lancet*, 1905. *Scientific Memoirs, Government of India*, No. 35.

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A METHOD FOR DETERMINING THE ALKALINITY OF THE BLOOD

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Apart from the question whether the blood is really alkaline in the sense that it contains such an excess of hydroxyl ions as can be determined by the available physical methods, there is no doubt that the amount of acid which must be added to blood to produce a certain effect with a given indicator varies under different circumstances, and it seems clear that these variations are the expression of phenomena which cannot be neglected. The current methods of determining the alkalinity of blood involve the use of various coloured indicators—litmus, dimethylamidoazobenzol and the like. Since the effect which is produced by the addition of acid to blood is under these circumstances a change of colour, such processes are practically restricted in their application to plasma or serum. This would probably be on the whole rather an advantage for most purposes if the methods were in themselves precise. It seems to us, however, to be very difficult by these means to reach an exact and definite end-point. The ordinary coloured indicators do not give a sharp end-point when used in the titration of proteid solutions. In the first place the actual colour of the indicator may vary materially with the presence or absence of proteid. Serum + congo red will not turn blue after the addition of an enormous excess of strong mineral acid[1]. If two similar quantities of alkali, to one of which is added a little serum or egg-albumin, are titrated with acid in parallel using dimethylamidoazobenzol, it will be seen that at no stage in the process are the tints of the two solutions precisely the same, and it is impossible with any excess of acid to realise in the proteid solution the full red tint of the acid aqueous solution. In the second place, making such allowance as one can for these differences in tint, one finds that the end-point in the presence of proteid ceases to be sharp and is dragged out over a long range of added acid. Thus with dimethylamidoazobenzol

1. Gamble, *Journal of Pathology*, Vol. XI. p. 134, 1906; G. Mann, *Physiological Histology*, p. 455, 1902.

10 c.c. N/10 Na_2CO_3 + 2 c.c. of 20 per cent. solution of egg white required 11·9 c.c. N/10 H_2SO_4 to produce the first trace of pinkness and 14·8 c.c. to give a definite red, the end-point extending by indefinite gradations over a range of 2·9 c.c. N/10 acid or about 20 per cent. of the amount of acid used. Similar experiments with dimethylamidoazobenzol and $NaH CO_3$ gave a range of 3·2 c.c., with NaH_2PO_4 2·9 and 2·8 c.c., whereas without the addition of proteid the same titrations had an end-point range of 0·3 or 0·4 c.c. at most. The indefinite character of the end-point is sufficiently obvious with litmus in using the original method of Wright[1] and with dimethylamidoazobenzol in the modification introduced by Moore and Wilson[2]. With these methods the difficulty of saying where the colour of the test drop changes is increased by the fact that the periphery of the drop may be one colour and the centre another. The result is that the determination of the end-point is essentially subjective. That this does not necessarily vitiate the methods is of course clearly shown by the concordant data which have been obtained by their use[3]. But it seems to us that with these, as with other methods of a similarly subjective nature, consistent results can only be obtained by a practised observer, and that any isolated observations made when one's 'eye is out' are not altogether free from suspicion of error.

The possibility of dispensing with foreign indicators altogether was suggested by the method of Dare[4] in which acid is added to diluted laked blood until the absorption bands of oxyhaemoglobin are no longer visible on spectroscopic examination. In its original form this method, as was pointed out by Gamble[5], gives very vague results and is quite unworkable. But, as we showed some time since[6], if the temperature at which the reaction takes place is raised, pretty definite end-points are given within a reasonable time by changes in the colour of the haemoglobin and in the gross appearance of the mixtures of dilute blood and acid.

1. *Lancet*, 1897, Vol. II, p. 719.
2. *Bio-Chemical Journal*, Vol. I, p. 297, 1906.
3. Moore and Wilson, *loc. cit.*; W. N. W. Watson, *Journal of Pathology*, Vol. XII, p. 429, 1909.
4. *Philadelphia Medical Journal*, Vol. XI, p. 137, 1903.
5. *Journal of Pathology*, Vol. XI, p. 135, 1906.
6. *Guy's Hospital Reports*, Vol. LX, p. 211, 1906

The Flocculent Precipitate Reaction

A series of small test-tubes are prepared containing quantities of N/1000 sulphuric acid rising by 0·1 c.c. from 0·0 to 1·2 c.c., the total volume of liquid in each being made up to 2 c.c. with distilled water. Each tube then receives a drop (*circa* 0·02 c.c.) of blood; the contents are mixed and the tubes placed in a water bath at 45° C. for one hour. They are then removed, wiped clean and dry on the outside and examined. The earlier tubes—i.e., those containing 0·1 and 0·2 c.c. N/1000 acid—appear little different from the control tube containing no acid; careful examination shows, however, that they are faintly opalescent. The liquid in the next few tubes is definitely opalescent, and the density of the opalescence increases progressively with increasing quantities of acid. With average normal human blood, one of the tubes containing between 0·7 and 0·9 c.c. N/1000 acid shows a further change in that the liquid is more or less occupied by a coarse flocculent precipitate. The coherence of the individual flocculi is so slight that by gently inverting the tube two or three times they are distributed into a uniform opalescence. In the great majority of cases the appearance of this precipitate occurs sharply and definitely, and affords an obvious objective end-point of the titration. It is very seldom that there is any doubt as to the tube in which the flocculent precipitate is added to the opalescence.

We are accustomed to take as the actual end-point the point half-way between the last tube which does not, and the first tube which does, show the precipitate. Increased accuracy is obtained by doing each experiment in triplicate: the precision of the reaction is such that the minute errors involved in measuring the acid and the blood sometimes result in some of the three tubes which are supposed to contain just the same mixture giving a positive result, while in the remainder no precipitate is produced.

It is convenient to distribute the blood along the series of tubes with a small dropping pipette. With an outside diameter at the point of about 1·2 mm., a drop of normal human blood is rather more than 0·02 c.c. The exact volume is determined for each experiment by weighing five or ten drops and from the known specific gravity. Since the size of drop increases very rapidly as coagulation becomes imminent, it is desirable to take a series of drops for weighing both before and after distributing the blood in the test tubes, and to make the collection and

distribution of the blood as expeditiously as may be. To ensure uniformity among the drops, the requisite amount of blood should be collected from the finger or ear in a small glass pot before it is taken into the pipette. There is a curious difference in the size of the drops which the same pipette will deliver from the blood of different individuals, all the samples showing the same content in haemoglobin. If the blood has been obtained quickly, and is altogether free from any suspicion of clotting, the variation in the size of each drop with different samples of normal blood containing approximately the same percentage of haemoglobin is not more than about 0·002 c.c., so that for many purposes, using a well-standardised pipette, weighing may be dispensed with. If one also knows approximately the answer which is to be expected, the amount of blood required may be still further reduced, and six drops (0·15 c.c.) will suffice.

Knowing the volume of the drops, the amount of acid required may be conveniently expressed either in terms of a standard drop of 0·02 c.c. or as c.c. of N/10 acid per 100 c.c. of blood. Thus with drops of normal blood weighing 0·0243 gms. = 0·023 c.c., none of three tubes containing 0·7 c.c. N/1000 H_2SO_4 showed a precipitate which was present in two of three tubes containing 0·8 c.c. and in all three tubes containing 0·9 c.c. acid. The average amount of acid required was therefore 0·783 c.c. This is equivalent to 0·681 c.c. N/1000 acid for 0·02 c.c. blood or 34 c.c. N/10 acid per 100 c.c. blood.[1] An error of 0·1 c.c. in the amount of acid required or of 0·003 c.c. in the volume of the drop of blood makes a difference of 5 c.c. N/10 acid per 100 c.c. of blood, or about 170 c.c. for the whole blood.

The end-point is ultimately the same at whatever temperature between 15° and 45° the reaction takes place. At the lower temperature, however, the flocculent precipitate takes much longer to appear and the end-point is not so sharply marked. At 34° for example it takes two hours, at 20° three hours to reach the same point as appears at 45° in half an hour. The flocculent precipitate is well marked at 45° in about thirty minutes, often in much less, and no alteration of the point at which it occurs takes place during the next four or five hours. The period of one hour at 45°, which we take as the standard time, is therefore sufficiently remote from the stage at which any rapid change occurs.

1. In the terminology which has often been used for the alkalinity of blood this is equal to 136 mgms. NaOH per 100 c.c. blood.

The concentration of the acid, or the total volume to which the mixtures are made up, is, within wide limits, immaterial. Thus:—

Total volume of mixture		c.c. of N/1000 acid required Blood A	Blood B
1 c.c.	...	0·350	0·650
2 c.c.	...	0·367	0·650
3 c.c.	...	0·383	0·650
4 c.c.	...	0·350	0·650

Comparison of the different tubes is, however, facilitated if they are all about the same concentration, and we have found a total volume of 2 c.c. a convenient standard.

The nature of the flocculent precipitate. The reaction is given equally well by fresh, defibrinated, oxalated or citrated blood and by red corpuscles washed many times with salt solution. It is not given by citrated or oxalated plasma, by serum or by a solution of fibrin. If well-washed red cells are laked by adding distilled water equal in volume to the original plasma, and drops taken forthwith, a good reaction is obtained. But if the laked red cells are thoroughly centrifugalised, the supernatant fluid gives no reaction. If, on the other hand, the washed corpuscles are laked with N/100 Na_2CO_3, the supernatant fluid after centrifugalisation gives a fair flocculation, though not such a good reaction as before centrifugalisation. The reaction therefore depends on the presence of some part of the formed elements of the blood which does not go into solution on laking with distilled water. That the leucocytes are not concerned is probably shown by the fact that an emulsion of leucocytes free from red cells collected out of citrated blood gives no reaction. To be sure, however, that the reaction is not due to something in the leucocytes acting in conjunction with something in the red cells, it would be necessary to show that it is given by red cells free from leucocytes: this cannot be done, though the reaction seems to be just the same after as many leucocytes as possible have been removed. The precipitate is soluble in alkali or in excess of acid, about 1·4 c.c. N/500 acid being required for a drop of 0·02 c.c. The presumption, therefore, is that the precipitate is actually composed of the nucleo-proteid of red cells. It seems, however, at least likely that the characteristic flocculation is in part connected with the presence of particles (stromata) on to which the precipitation occurs.

Test experiments on blood—human or rabbit defibrinated[1]—to which known acid or alkali had been added—gave fairly satisfactory results:—

c.c. N/1000 H_2SO_4 required for 0·02 c.c. blood

Lactic acid added			KOH added			Na_2CO_3 added		
Calculated	Found	Diff. %	Calculated	Found	Diff. %	Calculated	Found	Diff. %
−0·149	0[2]	—	0·556	0·583	+4·9	0·447	0·450	+0·6
0·011	0[2]	—	0·709	0·717	+1·1	0·554	0·583	+5·2
0·270	0·250	−8·0	1·045	0·999	−4·6	0·617	0·583	−5·5
0·572	0·617	+7·3	1·235	1·249	+1·1	0·650	0·706	+8·6
0·713	0·717	+0·6				0·677	0·683	+0·9
						0·974	0·923	−5·5
						1·194	1·107	−7·8

It is clear that the method may be extended to the examination of such fluids as blood serum by using blood or red cells as an indicator. In this way the distribution of the apparent alkalinity of whole blood between corpuscles and serum may be ascertained. It is then found that the serum in normal human blood accounts for from 1/10 to 1/5, generally about 1/7 of the total alkalinity as measured by this method; in other words the serum has an alkalinity of about 5 c.c. N/10 acid per cent. In comparing one blood with another, therefore, an accurate knowledge of the relative amounts of corpuscles and plasma is essential. Red cells washed repeatedly in salt solution become less and less alkaline as the washing proceeds.

It has been already indicated that the flocculation end-point treats added Na_2CO_3 as if it were alkali. But if defibrinated blood (which gives the same answer as the fresh blood from which it is prepared) is shaken up with CO_2, the acidity is much increased.

c.c. of N/1000 acid required for 0·02 c.c.

Defibrinated blood		Saturated with 5·6 % CO_2		Saturated with pure CO_2
0·750	...	0·650	...	0·250
0·483	...	0·317	...	0·083

Defibrinated blood which has been thoroughly pumped out in a vacuum gives the same result as before treatment. The difference between arterial and venous blood is shown in the following figures giving the results of simultaneous measurements on blood from the carotid and large veins of rabbits under ether anaesthesia:—

c.c. N/1000 acid for 0·02 c.c. blood.

Arterial		Venous
0·600	...	0·557
0·576	...	0·494
0·563	...	0·467
0·532	...	0·488
0·382	...	0·304

1. Human gives a sharper end-point than rabbit blood.
2. Flocculation occurred without addition of any acid.

The average difference, therefore, is about 3 to 3·5 c.c. N/10 acid per 100 c.c. blood. A number of experiments showed that drops of blood obtained in the ordinary way gave the same results as samples taken from a moderately obstructed vein in the arm immediately afterwards.

Examples of the results obtained

(1) The time of day at which the blood is examined may have a considerable influence on the result. On first waking in the morning the blood is always more alkaline than during the active hours of laboratory work: e.g.

	c.c. N/10 acid %		c.c. N/10 acid %
7.10 a.m. ...	45·2 ...	7.0 a.m. ...	38·0
10.40 „ ...	35·7 ...	9.50 „ ...	36·6
12.20 p.m. ...	35·7 ...	11.55 „ ...	34·1
1.30 „ ...	31·0 ...	12.40 p.m. ...	32·2
2.35 „ ...	35·0 ...	8.45 „ ...	36·6
4·50 „ ...	40·5 ...	— ...	—
7.30 „ ...	36·0 ...	— ...	—
9.20 „ ...	36·0 ...	— ...	—

(2) Short sharp exercise (running, turning a pump handle) sufficient to produce marked dyspnoea increases the acidity:—

Before exercise	Duration of work	Immediately after	½ to 1 hour after
*35·7, 31·0 ...	1½ min. ...	23·8 ...	31·0
34·5, 34·1 ...	1 „ ...	27·8 ...	30·9
31·3, 34·0 ...	3 „ ...	27·1 ...	32·7
34·6, 33·9 ...	2½ „ ...	30·2 ...	34·6
*33·2, 34·7 ...	2 „ ...	25·9 ...	34·7, 36·2
36·6, 34·1 ...	1 „ ...	27·7 ...	32·2, 38·0
*33·5 ...	2¾ „ ...	29·0 ...	36·4

In the experiments marked * Dr. J. H. Ryffel was the subject. The exercise was in all cases sufficient to produce a definite increase in the lactic acid in the urine[1]. In the last experiment he also estimated the lactic acid in three 20 c.c. samples of blood taken from a vein in the arm, and found quantities equivalent to 1·4, 7·9 and 1·8 c.c. N/10 acid per cent.; relatively these figures are in satisfactory agreement with our own obtained from the same samples.

Less violent muscular work, extending over a longer period, such as two hours' singles at tennis or walking eighteen miles in five hours, seems to have no effect which can be detected at the end of the exercise.

1. *Proceedings Physiological Society*, January, 1910.

(3) We have not been able to find that meals have any influence. In one experiment, for instance, in which Dr. E. L. Kennaway was good enough to help us, the following results were obtained, a large mixed meal being eaten from 1 to 2 p.m. with good appetite:—

E. L. K.			A. E. B.		
Time	c.c. N/10 acid %		Time	c.c. N/10 acid %	
11.5 a.m.	...	32·4	7.15 a.m.	...	39·4
12.10 p.m.	...	33·9	10.10 „	...	35·9
12.40 „	...	30·2	12.15 p.m.	...	34·4
2.0 „	...	29·6	2.5 „	...	31·7
2.40 „	...	31·6	2.45 „	...	33·9
3.15 „	...	27·4	3.25 „	...	34·5
4.10 „	...	28·9	4.5 „	...	32·4

(4) A rabbit required 0·95 c.c. N/1000 acid for 0·023 c.c. blood; it was then poisoned with 90 c.c. 2 per cent. phosphoric acid *per os*. Three hours afterwards only 0·55 c.c. were required, and an hour later, when it was moribund, only 0·45 c.c.

Colour Changes in Haemoglobin

A. It was our original intention to use for the end-point the first visible change (from red to yellow) which is produced by acid in the colour of the haemoglobin. To investigate this, the mixtures of blood and acid, after one hour at 45° C., are saturated with coal gas and centrifugalised until all opalescence and precipitate has been got rid of. Time and temperature are more practically important factors in this reaction than in the production of the flocculation: experiment showed, however, that no rapid change occurred in the neighbourhood of one hour if the reaction was going on at 45° C. To ascertain the end-point exactly it is necessary to examine the tubes in a good open light, and it is very desirable to have the density of tint the same throughout by using tubes of equal diameter and making the total volumes of liquid exactly the same. Under these circumstances it was found in a number of experiments that the change of colour took place at the same point in the series as the flocculation. The former end-point is rather difficult and troublesome to make out: it was accordingly abandoned in favour of the more obvious flocculation. It is sensitive to added free CO_2 in the same way.

B. For some reason or other it is easier to see the point at which successive additions of acid cease to make the haemoglobin any more yellow. With 0·02 c.c. of normal human blood, this point is reached

with about 1·8 to 2·0 c.c. N/500 acid. To obtain by this means an expression of the alkalinity of the blood, the amount of acid consumed in converting the haemoglobin must be deducted, and it was therefore necessary to ascertain how much acid was required to convert a definite amount of haemoglobin into the yellow stuff (which may or may not be acid haematin). Haemoglobin crystals were prepared by various simple combinations of laking and cooling from horse corpuscles. The oxygen capacity of the solutions prepared from the fresh moist crystals was determined colorimetrically with a standard Gowers-Haldane haemoglobinometer and the absolute amount of haemoglobin calculated on the assumption—probably invalid—that 1 gramme of haemoglobin combines with 1·34 c.c. oxygen. On the same assumption it was found that haemoglobin constituted about 90 per cent. of the total solid matter in the solutions. These solutions were examined in series of tubes containing increasing quantities of acid as if they had been blood, being kept at 45° C. for one hour. In five experiments we obtained the following results, each figure being the average of a number of determinations with different amounts of haemoglobin from each lot of crystals. The figures represent the number of c.c. of normal H_2SO_4 required to completely convert 1 gramme of haemoglobin into a yellow pigment in one hour at 45° C.

1·13
1·01
1·09
1·06
1·08

Average = 1·07 c.c.[1]

From this figure the amount of acid consumed in converting the haemoglobin in any drop of blood may readily be computed. If, for example, the reading on the Gowers-Haldane haemoglobinometer is 72 per cent., then 0·02 c.c. of blood requires:—

$$\frac{72 \times 18{\cdot}5 \times 0{\cdot}02 \times 1{\cdot}07 \times 1000}{100 \times 1{\cdot}34 \times 100} = 2{\cdot}13 \text{ c.c. N/1000 acid}$$

In other words, for every 10 per cent. of haemoglobin on the scale of the haemoglobinometer, the haemoglobin in 0·02 c.c. of blood requires 0·295 c.c. N/1000 acid.

1. This suggests an equivalent of about 950 for haemoglobin if it is a mono acid base.

THE EQUILIBRIUM BETWEEN VARYING CONCENTRATIONS OF ACIDS AND ALKALIES AND THE PROTEINS OF THE SERUM AND OTHER COLLOIDS, WITH A DISCUSSION OF THE NATURE OF COLLOIDAL REACTION OR ADSORPTION

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It was shown by Moore and Wilson[1] in 1906 that the serum proteins possess a relatively enormous power of taking up acid or alkali within narrow ratios of hydrogen and hydroxyl ionic concentration. Thus serum titrated with alkali to the neutral point to phenol-phthalëin, and then to the neutral point to an indicator such as 'di-methyl' or methyl orange, shows a difference indicating no less a concentration than 0·14 to 0·16 Normal. Outside these two limits of neutrality to the two coloured indicators the hydrogen or hydroxyl ion concentration swings up very rapidly compared to the rate of change within the limits. We may hence speak of a 'neutral zone' for the protein of the serum. The traverse of this zone amounts to about 0·15 N, and within this zone the reaction is nearly neutral. Outside of it in either direction the acidity in the one case or alkalinity in the other mounts very rapidly. Moore and Wilson drew attention to the curious and interesting fact, that this concentration in regard to acid or alkali of the proteins amounts to about an isotonicity with the entire inorganic salts of the serum.

The tonicity for all mammalian sera is very nearly constant, amounting to about that of a 0·9 per cent. solution of sodium chloride, that is to say to about 0·15 N; this close coincidence can scarcely be fortuitous, and would support the view that the figure 0·15 N indicates an average reactivity of the serum to crystalloids, and shows that under normal conditions the amounts of crystalloids in the serum are regulated by the combining or adsorbing powers of the serum colloids.[2]

1. *Bio-Chemical Journal*, Vol. I, p. 311, 1906.

2. Forster, and also Sollman, on other and more purely physiological grounds, have suggested and discussed such a combination between proteins and salts in the plasma.

See J. Forster, *Zeitsch. f. Biologie*, Vol. IX. p. 297, 1873; Torold Sollman, *Amer. Journ. of Physiology*, Vol. VIII, p. 155, 1903.

When for any reason more crystalloids are present than can be held in this feeblest type of union by the proteins present, these free crystalloids become adsorbed (or united in some form) by the kidney cells and excreted; and, contrariwise, if salts are abstracted by the tissues, so that colloids are free, then crystalloids taken up from the alimentary canal are attached by the serum colloids to restore equilibrium in this direction.

The type of chemical or physical union here existing is one of the most delicate and labile with which we are acquainted; although its actual existence is demonstrated both by these titration figures, and by the delicate balancing of the tonicity of the serum by the excretory cells and tissue cells so as to preserve an almost absolute constancy in plasma tonicity. For example, this union between the crystalloids and proteins has no effect either upon freezing point or conductivity, the serum crystalloids behaving in these respects exactly as if they were entirely free. Also, the union cannot resist a gradient of osmotic pressure, the salts diffusing away from the proteins quite readily when placed in an osmometer with distilled water outside.

In addition to the evidence of such a feeble combination or union of some sort between crystalloids and proteins, arising from the titration figures to the two sides of the neutral zone, and of the minute physiological regulation of the tonicity of the serum or plasma, we know also from the direct measurements of osmotic pressure in serum by the osmomoter[1] that the state of solution of the proteins varies with the concentration and nature of the electrolytes present in common solution with them. Now, such variations can only possibly arise if there exists some variable or labile form of union between the proteins and the crystalloids.

In the series of experiments described in the present paper, we have attempted, by a special method, to follow up the conditions of equilibrium between inorganic acids and alkalies and the serum proteins, at intermediate positions within, and just without, the neutral zone, and not merely at the two ends, as is done when the serum is titrated in presence of the proteins to a neutral point to a coloured indicator.

There are immense difficulties in the way of determining by the electrode methods or other physico-chemical methods the concentration of hydrogen and hydroxyl ionic concentration in such fluids as bio-chemists have to deal with, and especially at the natural points of reaction of such fluids. To begin with, such reactions lie near the neutral point, as they

1. Moore and Parker, *Amer. Journ. of Physiology*, Vol. VII, p. 261, 1902; Moore and Roaf, *Bio-Chemical Journal*, Vol. II, p. 34, 1907; Adamson and Roaf, *ibid.*, Vol. III, p. 422, 1908; Lillie, *Amer. Journ. of Physiol.*, Vol. XX, p. 127, 1907.

do not depart widely from the reactions of most good tap waters. Within this region it is well known that the hydrogen electrode method gives experimental variations in the ratio of hydrogen and hydroxyl ions amounting to over one-hundred fold (10^2), and hence in this region, apart from the qualitative fact that the reaction is about what would be termed neutral in ordinary parlance, the electrode method can teach us nothing.

The second difficulty lying in the way, and most troublesome when coloured indicators are utilized, is that of the actual presence of the proteins, which by behaving like very weak acids or bases just like the indicators themselves, so dull off the end points that it is almost impossible to titrate with any degree of accuracy.

If the acid or alkali solution could be brought by some method into contact and equilibrium with the protein, and could then be removed without having its equilibrium disturbed or taking up from or giving out to the protein in the process, we would possess a fluid removed from most of these practical drawbacks, that is, one in which hydrogen or hydroxyl ionic concentrations could be directly determined, or since it is now only a dilute solution in water of acid or alkali, it can be titrated, and the concentrations referred to well known determinations of these in such purely crystalloidal solutions.

This is the basis of the experimental method we have utilized in the present research.

It was shown by Moore and Parker, for the purposes of their research into the osmotic properties of proteins by the direct method, that the usual crystalloids, such as sodium chloride, etc., of the plasma, rapidly pass through parchment paper and attain equilibrium on the two sides within twenty-four hours.

This forms the basis of a method introduced by B. Moore for the estimation of crystalloids when in common solution with colloids which render direct titration impossible, and it has been used in this laboratory already for many purposes, as in the determinations of sugar in blood by Edie and Spence,[1] and in the study of distribution of electrolytes in the blood corpuscles and serum by Moore and Roaf.[2]

The principle of the method is very simple. A known amount of the serum or other colloidal solution in which the determination is to be made is taken and placed inside or outside of a sausage dialysis tube, on the other side is placed a known volume of the dialysing fluid. The whole is

1. *Bio-Chemical Journal*, Vol. II, p. 103, 1907.
2. *Ibid.*, Vol. III, p. 55, 1907.

left so long as preliminary experiments have indicated to be necessary for equilibrium to be established. On the basis that when equilibrium is established the concentrations of crystalloids are equal on the two sides of the membrane, it is easy to determine any given crystalloid such as phosphates, chlorides, or dextrose in the outer protein-free fluid, and from this to calculate what it originally was in the serum or blood. For example, suppose 25 c.c. of serum are placed inside and 75 c.c. of distilled water outside, and at the expiration of twenty-four to forty-eight hours the outer fluid shows on analysis of 50 c.c. by some accurate method, such as the gravimetric method, that this contains 0·02 gm. of sugar, then, since this is contained in 50 c.c., the total amount in the 100 c.c. is 0·04 gm., and as 25 c.c. of serum were taken the 0·04 gm. belongs to 25 c.c., and accordingly in 100 c.c. there would be $0{\cdot}04 \times 4 = 0{\cdot}16$ per cent. of sugar.

This example shows how the method may be applied for determining the normal amount of any given crystalloidal constituent of the serum.

For the purpose of determining equilibria between serum proteins and acids or alkalies, the method of calculation and procedure varies somewhat. Here a known number of cubic centimetres of decinormal acid or alkali is added to a known volume of serum or to the known volume of dialysing water placed outside, and as before a definite volume of the outer fluid is measured off and titrated after a sufficient time-interval for equilibrium has been allowed to elapse.

In the case of any particular experiment with serum, a series of dialyses were always run simultaneously with gradually increasing amounts of added acid or alkali in each tube.

The arrangement of the experiment was that always 10 c.c. of serum *plus* the desired number of cubic centimetres of decinormal acid (or alkali) were mixed and introduced into a sausage-shaped dialysing tube. Outside there was placed a sufficient amount of distilled water to bring up the total amount of fluid within and without to 100 c.c. in every case. After allowing forty-eight hours to elapse, which, as we shall see, is a quite sufficient period for the establishment of equilibrium, 50 c.c. of the outer dialysate were removed and titrated with 'di-methyl' as indicator.

The calculation of amounts of free acid and of acid in union with protein is exceedingly simple. For the concentration of free acid within and without the dialyser is the same, when equilibrium is completely established. Accordingly, the total volume being 100 c.c. by doubling the titer of the 50 c.c taken from outside, we get the total amount of free acid, and simply subtracting this from the total number of cubic centi-

metres of decinormal acid originally added to the 10 c.c. of serum inside, we get the amount expressed also as cubic centimetres of decinormal acid in union with the proteins of the serum.

The ratio is thus directly obtained of free and of combined or adsorbed acid—or, perhaps, simply and without theorising, it might be called 'united' acid.

We shall see, as found by van Slyke and van Slyke[1] for insoluble casein and acid, that this 'united' acid increases in amount with increase in acid concentration, and does so in no simple linear relationship to the concentration, so that *in van Bemmelen's sense of the term the process is one of adsorption.*

We shall endeavour, however, to show in our theoretical discussion that, in our opinion, this does not suffice to determine whether the action is a purely physical, surface, or solid solution one, or is a chemical reaction.

The experiments are very conveniently carried out in wide mouthed glass stoppered bottles, capable of holding about 150 c.c., and wide enough to take the parchment sausage tube used without folding laterally upon itself. It is well to sterilize both bottles and parchment tubing before commencing, and the bottles must of course be dry.

The glass stoppers inserted afterwards so as to hold the doubled top of the parchment paper between bottle neck and stopper serve to hold the parchment tubes in position, and at the same time prevent the ingress of dust. Two drops of 'di-methyl' added to the outer fluid serve to show the progress of the dialysis, and also for titration afterwards. In working with alkali, the indicator may be changed to phenol-phthalëin. The method is less satisfactory for alkali than acid, especially for summer work, as the contents of the tubes often decompose within forty-eight hours. This is never seen with acid-containing tubes, even in hot weather. The acid we have used has been decinormal sulphuric acid, but similar experiments can be performed with other acids.

Before commencing the dialysis experiments, we performed a number of simple titrations in presence of the proteins to get the limits of distinct colour change to 'di-methyl' and to phenol-phthalëin in relationship to the breadth of the neutral zone as given by Moore and Wilson's figures, and to get the tonicity figure we determined the depression of the freezing point of each sample of serum used so as to get a figure for the total crystalloidal content to compare with the titration figure. In regard to

1. *Amer. Chem. Journ.*, Vol. XXXVIII, p. 383, 1907.

these figures, it must be admitted that they cannot be taken as accurate within closer limits than about 0·5 c.c. at each end. Making every allowance for this, however, there is a most remarkable coincidence between the acid-alkali value of the proteins and the total crystalloidal value as shown by the freezing point. Neither values show much variation in the sera we have tested.

We shall first give the record of these results, then that of the dialysis figures.

A. Total reactivity of Serum Proteins, compared with total Crystalloidal Content

When the difficulties of determining the freezing point with sufficient accuracy for these purposes on the one hand, and of titrating to a coloured indicator in presence of proteins on the other, are considered, the comparative closeness of the estimates of the 'Normality' of the serum by the two methods as shown in Table I, p. 38, appears to us to be more than a coincidence.

The results given by the dialysis method (*vide infra*, p. 41) show the interesting fact that at the place where the reaction in the protein-containing fluid was just on the turn in colour to the indicator, there was a good deal of free acid present. This appears to us to indicate that there is probably some union between the dye of the indicator and the protein whereby the indicator is protected from the action of the acid until practically all the protein power for union with acid has been used up.

When we look at the figures obtained by the dialysis method, we see that if concentrations are avoided at which injury and permanent change is caused to the protein, then there is a limit attained at which further increase in acid concentration does not practically increase the amount of 'united' acid, and only the amount of free acid concentration goes on increasing.

After this range of almost constant amount of the 'united' acid has been passed, there is again a sudden increase in amount of 'united' acid. This break we believe to be due to slow permanent alteration of the proteins by action of the acid or alkali. We have neglected such observations with higher amounts of acid or alkali, as they are, in our view, due to quite a different cause. Such injury is more readily caused by alkali than by acid.

If the real maximum point of complete union of protein and alkali or acid be taken, and the two titration values to acid and to alkali be added

TABLE I

	Type of serum	Total reactivity of the proteins of sera. 10 c.c. of serum taken for titration: Acidity to di-methyl in c.c. N/10 H_2SO_4	+	Alkalinity to phenophthalein in c.c. N/10 NaOH	=	Total reactivity in c.c. N/10 conc.	=	Therefore proteins correspond to a normal concentration of	Total crystalloidality of same sera: Depression of freezing point Δ	Corresponding conc. of sodium chloride solution in percentage	Normality
Experiment	I—Pig ...	14·9 c.c.	+	2·3 c.c.	=	17·2 c.c.	=	0·172 N	0·64	0·97	0·167 N
„	II—Pig ...	13·9 c.c.	+	3·1 c.c.	=	17·0 c.c.	=	0·170 N	0·64	0·97	0·167 N
„	III—Pig ...	12·6 c.c.	+	3·2 c.c.	=	15·8 c.c.	=	0·158 N	0·59	0·90	0·155 N
„	IV —Sheep ...	10·6 c.c.	+	2·1 c.c.	=	12·7 c.c.	=	0·127 N	0·65	0·99	0·170 N
„	V—Sheep ...	12·3 c.c.	+	2·9 c.c.	=	15·2 c.c.	=	0·152 N	0·64	0·97	0·167 N
„	VI—Ox ...	11·3 c.c.	+	2·1 c.c.	=	13·4 c.c.	=	0·134 N	0·62	0·95	0·164 N
„	VII—Ox ...	11·0 c.c.	+	2·1 c.c.	=	13·1 c.c.	=	0·131 N	0·62	0·95	0·164 N
„	VIII—Pig ...	10·9 c.c.	+	2·7 c.c.	=	13·6 c.c.	=	0·136 N	0·64	0·97	0·167 N

together, it is interesting to note that results very similar to those obtained by the direct titration method are obtained for the total 'binding'[1] power of the proteins. They correspond again quite closely to the mean 'total crystalloidality' or tonicity of the serum.

Taking the mean of the maximum figures for combination from the data of the following Section (Section B), we obtain the following results:—

FIGURES OBTAINED FROM 10 C.C. OF SERUM IN EACH CASE

Combined acid, N/10			Combined alkali, N/10		
1. Pig's serum (V)	=	11·8 c.c.	5. Pig's serum (VI)	=	5·5 c.c.
2. Sheep serum (III)	=	11·8 c.c.	6. Sheep serum (IV)	=	5·5 c.c.
3. Ox's serum (III)	=	10·5 c.c.			
4. Ox's serum (IV)	=	11·7 c.c.			
Average	=	11·4 c.c.	Average	=	5·5 c.c.

Total reactivity = 11·4 + 5·5 = 16·9 c.c. N/10.

Therefore total reactivity to acid and alkali of the proteins = 16·9 c.c. in 10 c.c. = 0·169 N. Reactivity.

The depressions of freezing point for these several sera were as follows:—

		Δ
1. Pig's serum (V)	=	0·629
2. Sheep's serum (III)	=	0·544
3. Ox's serum (III)	=	0·615
4. Ox's serum (IV)	=	0·590
5. Pig's serum (VI)	=	(lost)
6. Sheep's serum (IV)	=	0·617
Average Δ	=	0·599

Translating this Δ into terms of concentration of an isotonic solution of the most abundant crystalloid of the serum, viz., sodium chloride, we found as our mean measurement for a 0·9 per cent. solution of sodium chloride a Δ of 0·596; this is sufficiently close to the above average to be regarded as identical.

Hence the result is reached that the average tonicity of our sera is equivalent to that of a 0·9 per cent. sodium chloride. Now the molecular weight of sodium chloride (23 + 35) is 58, so that 0·9 per cent. corresponds to a $\frac{9}{58}$ Normal or to a 0·155 Normal. This figure is to be compared with 0·169 Normal obtained above for the total uniting power of the proteins.

1. We have made use of such words as 'union,' 'united,' and 'binding,' to prevent any theoretical conceptions of 'combination' versus 'adsorption' interfering with the statement of our experimental results.

B. Determinations by the Dialysis Method of the Equilibria between Serum Proteins and Acid (Sulphuric) and Alkali (Sodium Hydrate) at Different Concentrations

These experiments were carried out in the manner described above. To 10 c.c. of the serum the amount of acid or alkali specified in the first column was added, the two were thoroughly mixed and placed inside the sausage tube. Outside were placed 100 c.c. of distilled water *minus* the volume of serum and added alkali or acid placed within, so that there were altogether, inside and out, 100 c.c. For example, if inside were 10 c.c. serum + 12 c.c. N/10 acid = 22 c.c., then outside there would be 100 — 22 c.c. = 78 c.c. of distilled water.

To the outside fluid two drops of indicator, 'di-methyl' or phenolphthalëin were added, and the whole was left stoppered as described for forty-eight hours. Then 50 c.c. of the outer fluid were taken and titrated to neutrality, this titer doubled gave free acid (or free alkali, respectively), and the doubled figure subtracted from the total amount initially added gave 'united' acid or alkali, respectively.

Re-dialysis experiments were made by setting up the tubes after each experiment with the same volume of distilled water outside. These experiments of re-dialysis served to show that the acid or alkali is not permanently fixed to the protein, but dialyses out when the concentration is lowered in the solvent. Otherwise they were parallel to the original experiments, so the figures for them are not repeated.

EQUILIBRIUM TIME

Experiment IX.—To determine time necessary to ensure complete equilibrium. For this purpose a series of tubes were taken just as in the other experiments, but instead of varying the amount of acid in each, a constant volume of 14 c.c. of N/10 acid was added in all cases to 10 c.c. of serum and 76 c.c. of distilled water outside, and then the different tubes were titrated as to content of acid in their outer fluids as above described at the following varying time periods from the commencement of the experiment:—

	Time interval from start	Number of c.c. of free acid shown by doubling titre
Tube No. 1 ...	12 hours ...	3·2 c.c.
„ No. 2 ...	15 hours ...	3·6 c.c.
„ No. 4 ...	19 hours ...	3·4 c.c.
„ No. 5 ...	24 hours ...	3·8 c.c.
„ No. 6 ...	36 hours ...	4·0 c.c.
„ No. 7 ...	48 hours ...	4·0 c.c.
„ No. 8 ...	72 hours ...	4·0 c.c.

It is obvious from these figures that equilibrium was complete at the end of thirty-six hours, and as in our experiments under absolutely similar conditions and with the same paper, the time interval allowed was forty-eight hours, we believe that it may be taken that in all cases equilibrium was complete when titration was carried out.

In this test experiment with a concentration of acid in the solvent of 4 c.c. of N/10 per 100 c.c., i.e. of 0·004 N acid, the amount taken up was accordingly 10 c.c. of N/10 acid, which again corresponds fairly closely with the figures given below in the actual experiments.

A second experiment on the same lines gave similar results.

EQUILIBRIA WITH ACID

Experiment X.—Pig's serum (V), containing proteins 7·33 per cent. (by Kjeldahl), Δ = 0·629. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	2·1 c.c.	...	5·9 c.c.
10 c.c.	...	2·5 c.c.	...	7·5 c.c.
12 c.c.	...	4·0 c.c.	...	8·0 c.c.
14 c.c.	...	4·9 c.c.	...	9·1 c.c.
(16 c.c.	...	7·7 c.c.	...	8·3 c.c.) ?
18 c.c.	...	6·6 c.c.	...	11·4 c.c.
20 c.c.	...	8·2 c.c.	...	11·8 c.c.
24 c.c.	...	12·2 c.c.	...	11·8 c.c.

The figures are c.c. of N/10 H_2SO_4. It is seen that saturation of the proteins occurs with between 18 and 20 c.c. of added acid when concentration in the solution stands at about 7 c.c. of N/10 in 100 c.c or 0·007 N, and about 11·6 c.c. of N/10 acid have been taken up by the 0·733 gm. of protein in the 10 c.c. of serum. The titration at 16 c.c. is doubtful as to correctness, and is hence placed in brackets.

Experiment XI.—Sheep serum (III). Contained 6·85 per cent. of protein, Δ = 0·544. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	1·6 c.c.	...	6·4 c.c.
10 c.c.	...	2·2 c.c.	...	7·8 c.c.
(12 c.c.	...	2·6 c.c.	...	9·4 c.c.)
14 c.c.	...	4·4 c.c.	...	9·6 c.c.
16 c.c.	...	4·8 c.c.	...	11·2 c.c.
18 c.c.	...	6·2 c.c.	...	11·8 c.c.

Experiment XII.—Ox serum (III). Contained proteins, 7·49 per cent., Δ = 0·615. Equilibria to acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	3·2 c.c.	...	4·8 c.c.
(10 c.c.	...	4·8 c.c.	...	5·2 c.c.)
12 c.c.	...	4·4 c.c.	...	7·6 c.c.
(14 c.c.	...	3·6 c.c.	...	10·4 c.c.) ?
16 c.c.	...	5·4 c.c.	...	10·6 c.c.
18 c.c.	...	7·6 c.c.	...	10·4 c.c.

In this experiment the results are somewhat irregular, but a maximum is reached here also at a low concentration.

Experiment XIII.—Ox serum (IV). Proteins 7·03 per cent., Δ = 0·590. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	1·8 c.c.	...	6·2 c.c.
10 c.c.	...	2·2 c.c.	...	7·8 c.c.
12 c.c.	...	2·8 c.c.	...	9·2 c.c.
14 c.c.	...	4·2 c.c.	...	9·8 c.c.
16 c.c.	...	5·0 c.c.	...	11·0 c.c.
18 c.c.	...	6·4 c.c.	...	11·6 c.c.
20 c.c.	...	8·2 c.c.	...	11·8 c.c.

Here also a maximum union is obtained at about 16 c.c. of added acid with about 11 c.c. in combination, concentration of acid for maximum union about 0·005 N.

Experiment XIV.—Sheep serum (VI).

Total acid added		Free acid		United acid
6 c.c.	...	1·4 c.c.	...	4·6 c.c.
8 c.c.	...	1·6 c.c.	...	6·4 c.c.
10 c.c.	...	2·4 c.c.	...	7·6 c.c.
12 c.c.	...	3·0 c.c.	...	9·0 c.c.
14 c.c.	...	4·4 c.c.	...	9·6 c.c.
16 c.c.	...	5·4 c.c.	...	10·6 c.c.
18 c.c.	...	7·4 c.c.	...	10·6 c.c.

Result much as in preceding experiments, complete uptake of between 10 and 11 c.c at about 5 c.c. of free N/10 acid in 100 c.c., i.e., at 0·005 N concentration.

EQUILIBRIA WITH ALKALI

Experiment XV.—Pig serum (VI). Proteins 6·60 per cent. Equilibria with alkali (N/10 NaOH).

Total alkali added		Free alkali		United alkali
2 c.c.	...	0 c.c.	...	2 c.c.
(3 c.c.	...	1 c.c. ?	...	2 c.c.)
4 c.c.	...	1·6 c.c.	...	2·4 c.c.
6 c.c.	...	3·4 c.c.	...	2·6 c.c.
8 c.c.	...	4·4 c.c.	...	3·6 c.c.
10 c.c.	...	5·6 c.c.	...	4·4 c.c.
12 c.c.	...	6·8 c.c.	...	5·2 c.c.
15 c.c.	...	9·2 c.c.	...	5·8 c.c.

It is to be observed that the uptake of alkali is less than that of acid, amounting when the full amount is reached to less than 6 c.c., and requiring a somewhat higher concentration of alkali (0·007 to 0·009 N) to hold it in union with the protein.

Experiment XVI.—Sheep serum (IV). Proteins 6·87 per cent., Δ = 0·617. Equilibria with alkali.

Total alkali added		Free alkali		United alkali
2 c.c.	...	1 c.c.	...	1 c.c.
4 c.c.	...	2·2 c.c.	...	1·8 c.c.
8 c.c.	...	4·6 c.c.	...	3·4 c.c.
12 c.c.	...	6·6 c.c.	...	5·4 c.c.
15 c.c.	...	9·4 c.c.	...	5·6 c.c.

Here again the important result is that there is full uptake at about 12 c.c., with about 5·5 c.c. taken up and 6 to 7 c.c free (i.e., in 0·006 to 0·007 N).

The main experimental results of this series of experiments may be summarised as follows:—

1. Serum proteins of the mammalia are capable of taking up a very considerable amount of alkali or acid when there is a certain concentration of acid or alkali kept up.

2. Even with the first addition of acid or alkali some free acid or alkali is left in solution; this is usually taken as a criterion of adsorption. In van Bemmelen's sense these are adsorptions, but in our opinion molecular chemical union occurs.

3. The amount taken up varies with the concentration of free acid or alkali in the solvent, but a maximum is reached in both cases above which practically no more is taken up with considerable increase in concentration, short of the amount necessary to injure the protein.

4. The acid or alkali concentration required to maintain almost complete union is a very low one, amounting to about 0·006 to 0·007 Normal.

5. The amount of acid taken up is about double that of alkali. Ten c.c. of serum take up about 11 c.c. of N/10 acid, and about 5·5 c.c. of N/10 alkali.

6. If the average amount of serum protein be taken in the experiments at 7 per cent., the above figures mean that 0·7 gm. of protein in the 10 c.c. take up 11 c.c. of N/10 acid or 5·5 of N/10 alkali respectively. The combining weight of the protein, supposing one of protein to unite with one only of acid or alkali, corresponds to that amount which neutralises 10,000 c.c of N/10 acid or alkali. This gives approximately 640 for acid and 1,280 for alkali. Now the osmotic equivalent of serum proteins, as determined by direct measurement in the osmometer, amounts to about 40,000. So that this indicates that each solution aggregate of protein

can unite with about sixty molecules of acid or thirty molecules of alkali.[1]

7. The interval between the limits of uniting power of protein for alkali and acid in the two directions of acidity and alkalinity correspond approximately with the *total* amounts of crystalloid contained in the plasma, indicating a labile union between crystalloids and serum proteins.

8. This interval may be defined as the 'neutral zone' of the plasma.

C. Equilibria of Casein Solutions with Acid and Alkali

The above method of measuring uniting power of serum proteins was also applied in a few experiments to casein suspensions. The 'adsorbing' power of casein for acids has been carefully determined by the van Slykes[2] in a method which in principle consists in leaving casein in contact with acids of the definite concentrations and then filtering off from the insoluble casein and taking the electrical conductivity of the filtrate. The filtrate contained small amounts of protein and could not be titrated directly with accuracy. In the present method this inconvenience is entirely obviated, for we found on careful testing that the dialysate was always protein free. Hence the dialysate could be titrated after reaching equilibrium in the ordinary way.

Experiment XVII.—Casein emulsion (I). Containing 9 per cent. of casein as precipitate. Equilibria to acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
3 c.c.	...	0·6 c.c.	...	2·4 c.c.
6 c.c.	...	1·8 c.c.	...	4·2 c.c.
9 c.c.	...	3·2 c.c.	...	5·8 c.c.
12 c.c.	...	5·8 c.c.	...	7·2 c.c.
(15 c.c.	...	8·2 c.c.	...	6·8 c.c)
18 c.c.	...	10·6 c.c.	...	7·4 c.c.
21 c.c.	...	13·6 c.c.	...	7·4 c.c.

It is seen here again that free acid is present from the beginning and that there is a limit of union at about 12 c.c. of added acid with a concentration of free acid of about 6 c.c. (i.e., a concentration of 0·006 N), and with about 7 c.c. of acid taken up by the casein.

Experiment XVIII.—Casein solution (II). Containing 9 per cent. of casein. Equilibria to alkali (N/10 NaOH).

Total alkali added		Free alkali		United alkali
3 c.c.	...	0 c.c.	...	3·0 c.c.
6 c.c.	...	0 c.c.	...	6·0 c.c.
9 c.c.	...	0·8 c.c.	...	8·2 c.c.
12 c.c.	...	2·0 c.c.	...	10·0 c.c.
15 c.c.	...	3·8 c.c.	...	11·2 c.c.
18 c.c.	...	5·0 c.c.	...	13·0 c.c.
21 c.c.	...	7·0 c.c.	...	14·0 c.c.

1. The importance of this point, that each solution aggregate of protein possesses at the saturation point the power of uniting with as many as 60 molecules of acid is pointed out later in discussing the affinity constant of the colloidal reaction.

2. *Loc. cit.*

A result somewhat different from that with acid is seen here, the casein taking up about 8 c.c. before there is an appreciable pressure of free alkali allowed to develop. This looks like a very stable union of casein and alkali as shown in other ways by many observers.

The curious result then follows that on adding more alkali there is a further uptake held in by alkali pressure and approaching a maximum just where the quantity in union has been doubled, i.e., where the amount in unstable union equals that more firmly held. This occurs with a pressure of alkali corresponding to 7 c.c. of N/10 in 100 or to 0·007 N concentration. These two results correspond probably with the two amounts of alkali taken up at the neutral point to litmus (neutral caseinate of Söldner) and that taken up at the neutral point to phenolphthalëin (basic caseinate of Söldner). The former is the amount taken up when dilute alkali and excess of casein are shaken up together and then separated by filter or centrifuge. The second is the body obtained when more alkali is added to the filtrate until it is just alkaline to phenolphthalëin. The second of these is not the double of the former, and our experiments show that it represents an artificial point, the true basic value being almost exactly double the neutral value. Since in shaking up with excess of casein all free alkali is taken up, it is clear that Söldner's neutral caseinate corresponds to our point of no free alkali. Moreover, Söldner found one gram of casein to take up 8 c.c. of N/10 alkali while we first get pressure of free alkali when 7·7 c.c. have been taken up.

D. Effects of Heat Coagulation upon the Equilibria of Serum Proteins with Acids or Alkalies

These experiments are interesting in view of the prevalent notion that such unstable unions are, by those who speak of physical adsorption, thought to depend on the amount of surface of the solid or colloidal adsorbing phase of the system.

If this were correct, then since boiling ought to diminish surface of colloid by causing coagulation, one might naturally expect less 'adsorption.' The experiment actually showed that more was taken up in the boiled condition, but shelter against this might be taken by those holding the physical side, admitting that, after boiling, a certain amount of the acid went into true chemical union, either with the protein or with some hydrolytic products split off from it.

In any case, here follow the experimental results, obtained by the usual method, as above.

Experiment XIX.—Equilibria with alkali.

Pig Serum

	Boiled		Unboiled (Same sample)	
Total added	United	Free	United	Free
3 c.c. ...	2·4 c.c. ...	0·6 c.c. ...	2·0 c.c. ...	1·0 c.c.
4 c.c. ...	3·0 c.c. ...	1·0 c.c. ...	2·4 c.c. ...	1·6 c.c.
6 c.c. ...	4·8 c.c. ...	1·2 c.c. ...	2·6 c.c. ...	3·4 c.c.
8 c.c. ...	6·0 c.c. ...	2·0 c.c. ...	3·6 c.c. ...	4·4 c.c.
12 c.c. ...	8·0 c.c. ...	4·0 c.c. ...	5·2 c.c. ...	6·8 c.c.
14 c.c. ...	9·4 c.c. ...	4·6 c.c.		

The boiled samples, it will be seen, not only throughout hold more in union at a lower pressure but a maximum is not even attained with 9·4 in union. This probably means considerable hydrolysis followed by stable chemical union.

Experiment XX.—Equilibria with acid.

Pig Serum

	Boiled		Unboiled (Same sample)	
Total acid added	United acid	Free acid	United acid	Free acid
10 c.c. ...	0·8 c.c. ...	2 c.c. ...	7·4 c.c. ...	2·6 c.c.
12 c.c. ...	— ...	(lost) ...	7·9 c.c. ...	4·1 c.c.
14 c.c. ...	— ...	(lost) ...	9·1 c.c. ...	4·9 c.c.
16 c.c. ...	9·8 c.c. ...	6·2 c.c. ...	8·3 c.c. ...	7·7 c.c.
18 c.c. ...	11·0 c.c. ...	7·0 c.c. ...	11·4 c.c. ...	6·6 c.c. ?
20 c.c. ...	11·2 c.c. ...	8·8 c.c. ...	11·8 c.c. ...	8·2 c.c.

Here the quantities free and united are about the same in the two cases, boiled and unboiled, and any slight difference there may be is in favour of the unboiled specimens.

E. Other Colloidal Solutions

In order to further test the question of amount of colloidal surface at an interface between colloidal aggregate and solvent, having an influence on this equilibria of acid or alkali with serum proteins, a few experiments were instituted with other suspensions and colloidal solutions.

The results which were small or negative showed conclusively that this uptake of acid or alkali is a specific thing, only occurring when there is something of the nature of affinity between colloid and acid or alkali.

For example, animal charcoal in suspension which possesses such a high power of taking up and binding organic coloured bodies possessed no attractions whatever for acid in solution, and certainly no acid was taken up.

The suspensions or solutions tested were as follows:—

1. Animal charcoal, 5 per cent. suspension in water. Reaction neutral.
2. Starch paste, 1 per cent. paste.
3. Gum acacia, 5 per cent. solution.
4. Colloidal platinum suspension (Bredig).

The experiments were carried out as above described, 10 c.c. of each of the above being treated with 10 c.c. of N/10 H_2SO_4 and dialysed against 80 c.c. of distilled water outside. It was found that the starch, charcoal, and colloidal platinum showed no appreciable uptake of acid. Gum acacia in two different experiments gave a union of 2·4 c.c. and 3·0 c.c. respectively.

A Short Review of Related Literature

The literature on the subject of crystallo-colloids stretches far into the past, for ever since proteins have been isolated and analysed it has been known that they contain in all cases crystalloids in some intimate relationship, so that they cannot be freed from these without undergoing important alterations in their physical, chemical and physiological properties. Much controversy has been waged as to whether this so-called ash of the protein is an impurity or an integral part of the molecule, and it is only recently that physical experiments in osmotic pressure variations associated and related to crystalloidal changes have brought what may perhaps be regarded as a final proof that the crystalloid forms not an impurity, but rather a central and essential part of the colloidal aggregate or protein molecule.

It is impossible to quote here the whole of this extensive literature, and we intend only to refer to a few of those papers which have a special bearing on the present experiments. Many papers we may exclude which refer only to insoluble forms of protein saturated with acid or alkali, or to protein salted out of solution. The main problem we are here dealing with is protein in equilibrium with acid and alkali at varying concentrations of these reagents; this we have been able to investigate by the dialysis method. So far as we are aware, dialysis has not been used before for this precise purpose of inducing an equilibrium at definite acid and alkaline concentrations, by other observers. Dialysis has been used by Hardy[1] in the case of globulin, to follow the 'grade' of solution

1. *Journ. of Physiology*, Vol. XXXIII, p. 251, 1905.

corresponding to different acid concentrations. This observer has also used the methyl acetate inversion method to study the degree of ionization of acid in presence of globulin, but so far as we know he has not calculated the amounts of free and united acid in equilibrium at varying concentration. Nor would the methyl acetate method lend itself so well as dialysis to such a purpose, since we possess no guarantee that the united acid or alkali would not aid the free acid or alkali in attack on the substrate.

Some of the earlier results on the composition of the crystalline proteins formed by combination between crystalloids and various proteins are very interesting, in view of the results we have above obtained for the ratios of crystalloid (acid or alkali) and protein at the saturation point. Thus, Schmiedeberg,[1] that great pioneer of protein chemistry, obtained in 1877, beautifully formed glistening polyhedral crystals of a compound of magnesium oxide with a globulin of vegetable origin from Brazil nuts (*Bertholletia excelsa*). This crystalline magnesium-globulin compound of Schmiedeberg was examined and analysed by Drechsel,[2] who found that the compound dried at 110° C. contained 1·40 per cent. of MgO, which leads to a molecular weight of 2817 on the assumption of one atom of magnesium in the molecule. Drechsel also improved the technique for obtaining this compound by introducing dialysis with alcohol, and now obtained a molecular weight of 2757. Also, Drechsel was able by dialysis with alcohol outside to obtain the corresponding sodium oxide compound, which was found to contain 3·98 per cent. of Na_2O, leading to a molecular weight of 1496.

This figure is worth comparing with the figure calculated on similar lines for the sodium hydrate compound with serum proteins, existing at saturation point, with a free concentration of alkali in the solvent of about 0·007 Normal NaOH. At about saturation in Experiment IV, 10 c.c. of a 6·6 per cent. protein solution holds in union 5·2 c.c. of N/10 sodium hydrate. That is to say, 0·66 gm. of protein corresponds to 5·2 c.c., therefore as a gram-molecule of sodium hydrate is contained in 10,000 c.c. of N/10 alkali solution, we have as the weight of protein combining with this $\frac{10{,}000}{5{\cdot}2 \times 0{\cdot}66} = 1{,}260$ to 1280. This figure, therefore, would represent the mean molecular weight of the serum protein, on the supposition that one molecule of protein combines with one molecule of sodium hydrate. As has been pointed out above, direct osmometric

1. *Zeitsch. f. physiol. Chemie*, Bd. I, S. 205, 1877.
2. *Journ. f. prakt. Chemie*, N.F., Bd. XIX, S. 331, 1879.

measurements show that each solution aggregate combines with a considerable number of sodium hydrate molecules.

It is remarkable how closely of the same order are these older figures with more modern ones, such as our own, and others with casein, which will be mentioned later. It is also to be noticed that the figures for the divalent magnesium are about double those for the monovalent sodium.

The low values for the crystalloidal portion in such analyses caused at the time the results to be distrusted, and researchers turned towards regarding the absence of crystalloid as a test of the purity of proteins. This led to Harnack's work and the production of the so-called ash-free protein.

This substance caused much debate in its time, and it was pointed out that it had lost many of the usual native protein properties.

We now know from direct osmometric work that an ash-free protein can exist, but that it is an inert protein which has lost its solution properties and no longer shows osmotic pressure.

The purified pressure-free globulin of Weymouth Reid[1] is a modern congener of Harnack's pure ash-free protein, but the question in all probability is not one of purity, but one rather of the presence of crystallo-colloidal union or its absence.

Harnack[2] also prepared two distinct copper proteins from egg albumen, one containing twice as much copper oxide as the other. On the supposition of one copper atom, his figures lead to simplest molecular weights of 4696 and 2348. Loew[3] prepared two similar silver-proteins with percentages of silver closely corresponding to those of copper in Harnack's substances, and showing probably thereby that stochiometric relationships do hold in such cases. These two distinct pairs of compounds may be correlated with the two casein compounds mentioned later, as given by the work of more modern authors, and with the two values given by our Experiment XVIII. In this experiment we get no appreciable free alkali at 6·0 c.c. of added N/10 alkali, and a concentration of only 0·8 c.c. of N/10 in 100 c.c. (i.e., 0·0008 N concentration) with a total of 8·2 c.c. united. We shall, therefore, not go far out in assuming that appreciable free alkali is first present when 7 c.c. of casein are bound. Now, as 10 c.c. of 9 per cent. casein solution were taken, it follows that 0·9 gm. of casein unites, without free alkali pressure developing, with 7 c.c. of

1. *Journ. of Physiology*, Vol. XXXI, p. 438, 1904.
2. *Zeitsch. f. physiol. Chemie*, Bd. V, S. 198, 1881.
3. *Arch. f. d. ges. Physiol.*, Bd. XXXI, S. 402, 1883.

N/10 sodium hydrate. This leads, on a similar calculation to that given above, to a molecular weight of 1587 for the casein, with one molecule of NaOH to one protein molecule. On adding more alkali further uptake occurs, until a total of about 14 c.c. of N/10 sodium hydrate have entered into union before saturation is reached. Now this figure is just double the first, leading to a molecular weight of approximately 793 for a single molecule of sodium hydrate. The acid-caseinate compound, on the other hand, only forms under acid pressure, and becomes completed at about a pressure of 0·006 N, when the uptake is about 7 c.c. by 0·9 gm. of casein, corresponding to the first of the two alkali-caseinates.

These figures may be compared with those quoted by Söldner and many other observers using precipitation methods for acid caseinates and saturated uptake by alkali for alkaline caseinates.

We need hardly point out that we do not consider these as true molecular weights, but only as expressing the simplest stochiometric relationships of crystalloid and colloid at certain well marked points.

Many approaches, both by ordinary chemical analysis and by physico-chemical methods, have been made to the study of the interactions of proteins with acid and alkali.

Precipitation methods, followed by analysis of the acid-protein or alkali protein, have been utilised by Söldner,[1] Courant,[2] Paal[3], Spiro and Pemsel,[4] Cohnheim and Krieger,[5] Erb,[6] Osborne.[7]

Physical methods, such as conductivity determinations before and after protein additions, have been employed by Sjöqvist[8] and by Säckur,[9] and after removal of the acid-saturated protein by Lucius L. and Donald D. van Slyke.[10]

The electromotive force of concentration cells and the depression of freezing point have been utilised as methods by Bugarski and Liebermann;[11] Cohnheim[12] and also Hardy[13] have experimented by the

1. *Landwirthsch. Versuchsstat*, XXXV, p. 351, 1888.
2. *Arch. f. d. ges. Physiol.*, Bd. L, S. 109, 1891.
3. *Ber. d. deut. chem. Gesell.*, XXV, S. 1,202, 1892.
4. *Arch. f. physiol. Chemie*, S. 233, 1898-9.
5. *Zeitsch. f. Biol.*, XL, S. 95, 1900.
6. *Ibid.*, XLI, S. 309, 1901.
7. *Journ. of Physiol.*, XXVII, S. 398, 1901.
8. *Skand. Arch. f. Physiol.*, Bd. V, S. 277, 1895.
9. *Zeitsch. f. physikal. Chem.*, Bd. XLI, S. 672, 1903.
10. *Amer. Chem. Journ.*, Vol. XXXVIII, p. 383, 1907.
11. *Arch. f. d. ges. Physiol.*, LXXII, S. 51, 1898.
12. *Zeitsch. f. Biol.*, XXXIII, S. 489, 1896.
13. *Loc. cit.*

catalysis method for cane sugar and for methyl acetate, as first suggested by Ostwald.

All the authors practically who have used the above physical methods are agreed that proteins or protein salts both ionize and hydrolyse in aqueous solution; and experiments have been made by Hardy and others to determine both ionization and hydrolysis quantitatively.

There is, however, one matter about which care must be taken in dealing with such purely physical methods for determining or proving chemical union (or colloidal reaction or adsorption) between colloid and crystalloid, and that is that assumptions must not be made which amount to begging the question in interpreting the results. The strongest possible evidence appears to be accumulating that crystalloids can be in union with colloid without materially altering their separate physical properties, and hence physical properties measured after preparing solutions of colloid with crystalloid, or by adding crystalloids to colloidal solutions, may give very nearly additive functions in an independent way, although the crystalloids have united in an adsorption or 'colloidal reaction' with the colloid.

This has been recently shown by Moore and Roaf[1] in work upon the crystalloids of the serum and its own red blood corpuscles. The degree of interference with the freedom and physical properties of the crystalloid on its union depends to a large extent upon the amount of true specific chemical affinity and union between the two.

For example, the electrical conductivity and depression of freezing point of the serum are practically identical with those of its electrolytes, but the phosphates bound in with the haemoglobin of the red blood corpuscles do not show their full conductivity till fully detached. Also, in the case of globulins, neutral salts preserve their full conductivity, while the conductivity of acid and alkali in presence of globulin are very appreciably reduced.

In making conductivity and cryoscopic determinations we must therefore be careful of the proof that the values are due to a crystallo-colloidal compound, and not merely to crystalloid nearly free as to these properties, although anchored by colloidal affinities in the neighbourhood of the colloidal aggregates.

For example, there is good evidence, as we have shown, that the neutral salts of the serum are united to serum proteins, but while so

1. *Bio-Chemical Journ.*, Vol. III, p. 55, 1907.

united they produce full effects upon freezing point and conductivity just as if they were completely free. It would be obviously false, therefore, to calculate a mean molecular weight for the serum proteins from a Δ of the serum. This would be tantamount to assuming that each serum solution aggregate had only one ion attached to it, whereas we know, from taking in conjunction the freezing point depression and directly measured osmotic pressure, that each solution aggregate has many such ions attached to it. The same is illustrated by the experiments of this paper and the simple molecular weights deduced above are merely sub-multiples of the true ones showing the relative weight of colloid to each molecule of crystalloid.

The difficulties of accepting an ordinary salt formation and ordinary ionization are apparent in the classical paper of Hardy[1] on the globulins, which contains a large amount of accurate physical measurements and more clear reasoning than most of the papers dealing with this intricate subject by physico-chemical methods.

The author, abandoning his earlier view of a physical explanation, attempts a treatment of 'the phenomena of colloidal solutions in the 'special case of proteids from a frankly chemical standpoint.' He states that 'the chief difficulty in the way of a chemical theory of colloidal 'solution is the apparent need of postulating the existence of continuously 'varying chemical compounds, or what van Bemmelen calls absorption 'compounds.'

We agree with this author that 'such a need is more 'apparent than real.' The wild maze into which such a postulation of continuously varying chemical compounds leads may be appreciated by anyone who cares to read the series of long papers on the so-called 'ion-protein compounds' by T. B. Robertson.[2]

This theory is by T. B. Robertson usually ascribed to J. Loeb, but the whole later development has been the product of the pen of the former writer. Loeb merely introduced the word ion-protein to explain his and other people's results on cell stimulation by certain electrolytic ions. Such results had long before been obtained by Sidney Ringer, Howell and Locke; similar effects were noticed by Loeb some years later in increase of the percentage of parthenogenetic division in one or two species of

1. *Journ. of Physiology*, Vol. XXXIII, p. 251, 1905.

2. *Arch. f. d. ges. Physiol.*, Bd. CX, S. 610, 1905; *Journ. of Biolog. Chemistry*, Vol. I, p. 279, 1905-6, and subsequent volumes.

echinodermata[1] (falsely called artificial fertilization), and in some experiments upon rhythmical contractions induced in skeletal muscle by certain ions and inhibited by others.[2] It was clearly obvious to Ringer, Locke and others that calcium, potassium and phosphatic salts produced their effects by some type of union with protoplasm. Meanwhile the ionic theory of solution developed; Loeb stated that the ion in question unites with protein in some obscure way to form something upon which he fastened the tag ion-protein; then T. B. Robertson developed the so-called 'ion-protein theory,' and the net result has been that the real pioneer of the whole subject, who first developed the physiological or functional side of the study of the relations of crystalloids and colloids in living cells, namely, Sidney Ringer, remains known, as far as we can judge from their writings, to most of the workers of the Loeb school only as the author of 'Ringer's Solution.'[3]

1. It is only in species where parthenogenesis naturally occurs that it can be increased in amount by changes in tonic environment. For example, no artificial cell division can be induced by the procedures recommended by Loeb in the unfertilised eggs of *Echinus esculentus*. An altogether fantastic view has been taken of the importance of these 'artificial reproduction' experiments.

2. The rhythmical contractions of skeletal muscle induced by sodium chloride were well known to Ringer and published by him several years before Loeb re-discovered them (see papers numbered XI and XII in the list given below). Ringer also states that they were known and described by Biedermann. The antagonistic action of calcium and phosphates in arresting these contractions which has been discussed in several papers from the Loeb school, was also described in detail and discussed by Ringer in these much earlier papers.

3. [Note by B. Moore]. At some distant epoch it may happen that some American researcher, endowed with an extra measure of that antiquarian zeal which characterises his countrymen may dig out from musty archives of forgotten lore the history of 'Ringer's Solution,' and then that pioneer may at last be awarded the honour he has so long deserved but not received. In order to assist this research I append a list of the more important papers by Sidney Ringer, M.D., Professor of Medicine, University College, London, dealing with this important subject of the action of inorganic ions on living tissues. They may be recommended as studies of careful, accurate and painstaking research in a difficult and intricate subject:—

I. Concerning the influence exerted by each constituent of the blood on the contraction of the ventricle. *Journal of Physiology*, Vol. III, p. 381, 1880-2.

II. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *Journ. of Physiology*, Vol. IV, p. 29, 1883-4.

III. A third contribution regarding the influence of the inorganic constituents of the blood on the ventricular contraction. *Journ. of Physiology*, Vol. IV, p. 222, 1883-4.

IV. The influence of saline media on fishes. *Proc. Physiol. Soc.*, December, 1883; *Journ. of Physiology*, Vol. IV, p. 6.

V. An investigation regarding the action of rubidium and calcium salts compared with the action of potassium salts on the ventricle of the frog's heart. *Journ. of Physiology*, Vol. IV, p. 371, 1883-4.

VI. Concerning the influence of saline media on fish, &c. *Journ. of Physiology*, Vol. V, p. 98, 1884-5.

VII. On the mutual antagonism between lime and potash salts in toxic doses. *Journ. of Physiology*, Vol. V, p. 247, 1884-5.

VIII. An experimental investigation showing that veratria is similar to lime salts in many

To return to the globulins, Hardy states that 'though one may speak 'of the colloid particles as being ionic in nature, they are sharply distinct 'from true ions in the fact that they are not of the same order of 'magnitude as are the molecules of the solvent, the electric charge which 'they can carry is not a definite multiple of a fixed quantity, and one 'cannot ascribe to them a value, and their electrical relations are those 'which underlie the phenomenon of electrical endosmose. To such ionic 'masses I would give the name "pseudo-ions."'

It is obvious that Hardy's ion-proteins or 'pseudo-ions' are not the chemical complexes, 'ampholates' or 'ion-proteins' of T. Brailsford Robertson.

Hardy found that globulins unite in molecular proportions with both acids and bases, and finds some difficulty in explaining this. Combination of this type with acids could be explained, as he points out, on the analogy of the protein amino-acids to an ammonium base; but to explain combination with bases, the nearest analogy would have to be an acid anhydride such as CO_2 uniting with a molecule of sodium hydrate to form a bicarbonate. The behaviour with coloured indicators led Hardy to

respects as regards their action on the ventricle, also showing that veratria and lime salts are reciprocally antagonistic. *Journ. of Physiology*, Vol. V, p. 352, 1884-5.

IX. Regarding the influence of the organic constituents of the blood on the contractility of the ventricle. *Journ. of Physiology*, Vol. VI, p. 361, 1885.

X. A further contribution regarding the effect of minute quantities of inorganic salts on organised structures. *Journ. of Physiology*, Vol. VII, p. 118, 1886.

XI. Further experiments regarding the influence of small quantities of lime, potassium and other salts on muscular tissue. *Journ. of Physiology*, Vol. VII, p. 291, 1886.

XII. Regarding the action of lime, potassium and sodium salts on skeletal muscle. *Journ. of Physiology*, Vol. VIII, p. 20, 1887.

Note.—The rhythmic contractions of skeletal muscle re-discovered many years later by Loeb are all carefully described in these two papers in 1886 and 1887, as well as the effects of calcium and phosphatic ions in inhibiting them.

XIII. Concerning experiments to test the influence of lime, sodium and potassium salts on the development of ova and growth of tadpoles. *Journ. of Physiology*, Vol. XI, p. 79, 1890.

XIV. The influence of carbonic acid dissolved in saline solution on the ventricle of the frog's heart. *Journ. of Physiology*, Vol. XIV, p. 125, 1893.

XV. Further observations regarding the antagonism between calcium salts and sodium, potassium and ammonium salts. *Journ. of Physiology*, Vol. XVIII, p. 425, 1895.

XVI. The action of distilled water on Tubifex. *Proc. Physiol. Soc., Journ. of Physiology*, Vol. XXII, p. 14, 1897. See also Ringer and Buxton.

Concerning the action of small quantities of calcium, sodium and potassium salts upon the vitality and function of contractile tissue and the cuticular cells of fishes. *Journal of Physiology*, Vol. VI, p. 154, 1885.

Concerning the action of calcium, potassium, and sodium salts upon the eel's heart and upon the skeletal muscles of the frog. *Journ. of Physiology*, Vol. VIII, p. 15, 1887.

Upon the similarity and dissimilarity of the behaviour of cardiac and skeletal muscle when brought into relation with solutions containing sodium, calcium, and potassium salts. *Journal of Physiology*, Vol. VIII, p. 288, 1887.

See also Ringer and Phear. The influence of saline media on Tubifex Rivulorum. *Proc. Physiol. Soc.*, March, 1895, *Journal of Physiology*, Vol. XVII, p. 23.

The influence of saline media on the tadpole. *Journal of Physiology*, Vol. XVII, p. 423, 1894-5.

See also Ringer and Saintsbury, 'The Action of potassium, sodium, and calcium salts on Tubifex Rivulorum.' *Journ. of Physiology*, Vol. XVI, p. 1, 1894.

the view that the globulin may be regarded as having replaceable hydrogen atoms like phosphoric acid and the acid phosphates. If G. represents the globulin molecule, then the union of globulin with base, according to Hardy, might be expressed by:—

$$GH_n + NaOH = GH_{n-1}\,Na + HOH.$$

This leads to the great difficulty, in our mind, that only the sodium atom and not the hydroxyl ion unites with the globulin, and now on such a complex salt ionizing (since there might be 30 to 60 sodium atoms in each solution aggregate) the charge on the complex anion of colloid would be enormous. Also, the mechanism of union of acid on the one hand and base on the other would be quite different, a molecule of water being eliminated in the basic union, and a firm salt formed, while no elimination occurred in the acid union.

We can find no experimental evidence to justify the existence of such large ionic masses, as would require to be formed on Hardy's views, possessing surfaces much greater than the ordinary molecular dimensions and so forming a comparatively large interface charged by the presence of a large number (30 to 60) of similar ions with similar charges.

The velocity of the complex globulin ion was worked out by Hardy, by the boundary method of Whetham, and found to be of the same order as that for simple ions of the crystalloids. On Ostwald's law, an ion with so many atoms as the complex globulin ion ought to have an exceedingly low velocity, and Hardy points out that this law cannot hold above certain numbers of atoms in the ion, citing also other instances of departure from it.

It appears to us, however, that the ion postulated by Hardy is scarcely an ion at all in the ordinary sense of the term, and that Ostwald's deductions made for *increasing atomic number with constant ionic charge* cannot possibly be applied. It is obvious that the ionic velocity must decrease as number of atoms increases *if the ionic valency remains constant.* But if the ' pseudo ion ' becomes a mass defined by surfaces of dimensions outside molecular limits, then it is quite obvious that a different condition altogether has to be dealt with, in which proportionately to the increase of size of the aggregate the number of ionic charges increases also. If salt formation occurs, for example, between 60 molecules of acid and one solution aggregate of globulin, and then there follows free ionization, the aggregate will then have 60 ionic charges to pull it along in the electric field. Also, all those charges with the same sign will be brought closer together than in the solvent and be out of the region of opposite charges.

The cross section of the mass to be urged through the solution will be much less than the united cross sections of 60 ions; accordingly, instead of moving much more slowly than an ordinary crystalloidal ion, the 'pseudo ion' should move many times more rapidly.

The above reasoning may be illustrated by the difference in rate of falling of fog particles and raindrops. Gravitation takes the place of electric potential, the globulin is the dust particle around which the condensation occurs, the charged ions are the minute water particles of the fog, the raindrop falling rapidly through the air is the globulin 'pseudo-ion.' But no such acceleration on the one hand, nor the delay on the other due to size on the supposition of an ordinary charged ion, occurs; instead, the rate of movement is an ordinary ionic rate.

We would venture to suggest a much simpler view regarding conductivity of colloidal solutions and movements of colloids in the electric field, which so far as we can see fits with all the experimental facts.

Our view does not regard the colloids as amphoteric electrolytes, nor indeed as electrolytes at all, nor as having electric charges intrinsically belonging to them as ions, nor as carrying electric currents. The current is carried, in our view, entirely by the electrolytes of inorganic character, and when there are no such electrolytes there is no current and no colloidal movement. The colloids move because they are united to the crystalloids and must follow their movements, and they are precipitated at either electrode because they are there detached from their electrolytes.

Each solution aggregate when united to a crystalloid holds in colloidal union both ions of the crystalloid, that is to say both anion and kation. For example, globulin in neutral salt solution is not united only to sodium ions or only to chlorine ions, but to an equal number of sodium ions and of chlorine ions, and this number is a large one, say, thirty to sixty of each ion. Similarly, acid globulin is not a salt compound, nor does it hold in union hydrogen ions alone or the corresponding anions alone, but an equal and large number of hydrogen ions and anions. So also for alkali-globulin, an equal and large number of hydroxyl and of corresponding kations are held in union.

These colloidally united positive and negative ions are in some respects free, so that they can alter the freezing point and conductivity. Secondary alterations in ionisation due to the union with the colloid altering dissociation, and concentration of the ions in the neighbourhood of the colloidal aggregate and other such causes may occur, so that

conductivity may be considerably altered from that of the free crystalloid because of the presence of the colloid.

The ionisation of crystalloid still, however, remains high: much higher than direct ionisation of such colloidal bodies would probably give on any ordinary hypothesis of direct ionisation of a colloidal salt. Also these unions are of molecular kind between colloid and crystalloid and not atomic,[1] they are only kept up as long as there is a certain concentration of the crystalloid maintained in the solution, and there is no true atomic linkage with displacement of the elements of a water molecule, as in salt formation. The attraction is specific and hence chemical, and at the saturation point there are definite stochiometric relationships; but it is a union such as can occur between two atomically saturated bodies (such, for example, as a salt and its water of crystallization), and not an atomic union.

It is obvious that in such a union intermediate bodies with varying percentage composition can be separated off such as the adsorption compounds of van Bemmelen, and the varying amounts of bound acid or alkali shown by our experiments indicate such bodies; but this does not rule out chemical union nor debar stochiometric relationships. The most saturated product, however, will always possess a definite composition, corresponding to all points of attachment on the colloidal aggregates being occupied by crystalloidal molecules, and at this point more simple stochiometric relationships may be expected, as in our experiments above recorded, and in the haemoglobin compounds.

Also, the electrical conductivities and movements of the colloids in the electric field are easily accounted for on this view. For, *the movements of the colloids simply correspond to the algebraic sum of the movements of the two ions in union with them.* When, for example, globulin is dissolved in neutral salts, it is just as much in union with them as it is with alkali or acid when it is in solution along with these—why, otherwise, should they dissolve it? But, here the sodium ion and the chlorine ion move at about equal rates, the algebraic sum is zero, and there is no drift of colloid towards either electrode. When globulin is in acid solution, say in hydrochloric acid, the two ions in union with it in equal number are hydrogen and chlorine ions, the hydrogen is by far the more rapid of these two ions, and is moving towards the kathode. *Accordingly the whole crystallo-colloidal aggregate must move towards the kathode,* just as the concentration of the hydrochloric acid does, as shown by Hittorf, when it

1. This at once explains why the unions of globulin with acid and alkali occur between molecules and not atoms as found by Hardy.

is there alone in solution in water. On arrival at the kathode, the hydrogen ions of the crystallo-colloidal aggregate are discharged as hydrogen atoms, the chlorine ions are set free from the globulin and set moving backward towards the anode, and the globulin having now lost the acid with which it was united colloidally, is precipitated at the kathode. Turning to the anode, the chlorine ions arriving here are set free as chlorine atoms, attack water molecules by secondary decomposition, oxygen is set free, and hydrochloric acid is formed; this unites with more globulin and, again on account of greater velocity of hydrogen ion, the whole moves toward the kathode. In this way all the acid-globulin is finally conveyed towards and thrown out of solution at the kathode. It, itself, has not been an ion, has carried no electricity, and borne no charge, but its colloidal attachment to the hydrochloric acid has induced its movement. The explanation is exactly the same for alkali globulin, except that here the hydroxyl is the faster moving ion towards the anode, carrying globulin therefore in that direction and precipitating it there.

All cases of colloids, both organic and inorganic, united with crystalloids can be explained on such a basis. The colloid invariably is found to move in the same direction as the faster of the ions of the crystalloid with which it is colloidally united, and it is usually precipitated at the corresponding electrode from loss of its crystalloid, unless it is still soluble in its absence.

We have also considered closely all the inorganic colloids and their direction of movement in the electric field, and have found no exception to this rule. Starch and similar colloids which do not unite in colloidal reaction (adsorption) with crystalloids do not move in the electric field.

An important paper by Mellanby[1] in the same issue of the 'Journal of Physiology' as that containing Hardy's paper, adds a good deal of precise information to our knowledge of the properties of the globulins. The paper deals very exhaustively with the problems of solution and precipitation of globulins by neutral salts, alkalies and acids, from both theoretical and experimental standpoints. The author draws the following conclusions from his work:—'Solution of globulin by a neutral salt is due to forces exerted by its free ions. Ions with equal valencies, whether positive or negative, are equally efficient, and the

1. *Journ. of Physiology*, Vol. XXXIII. p. 338, 1905.

efficiencies of ions of different valencies are directly proportional to the squares of their valencies.'

'The amount of globulin dissolved by a given percentage of neutral salt is directly proportional to the strength of the original globulin suspension.'

'Solution of globulin by acids or alkalies is of the nature of a chemical combination. The relative solvent efficiencies of strong acids and alkalies are of the same order as their chemical avidities.'

'The precipitation of globulin from solution in neutral salts by neutral salts depends upon a molecular combination between the salt and globulin, the compound so formed being stable only in excess of the combining salt. Precipitation by salts of the heavy metals depends upon the formation of a stable globulin compound.'

These four conclusions of Mellanby, supported as they are by most abundant experimental evidence, appear to us to be of fundamental importance in the study of crystallo-colloidal combinations. Our own experiments, carried out by a different method, entirely agree with the greater part of them and confirm them, and we could wish no better support for the views which we hold as to the relationship of crystalloids and colloids.

In the first place the experiments, like our own, would conform to the van Bemmelen conception of adsorption compounds, but the author, like ourselves and most other authors, arrives at the conclusion that the process is a chemical one. This is now further confirmed by our result of a maximum uptake when a certain low concentration of the crystalloid has been attained.

The second result of Mellanby, that the uptake of globulin (colloid) in presence of the same percentage of saline (crystalloid) increases with original colloid concentration is the complement of the result of the van Slykes (vide infra) and ourselves, that the uptake of acid or alkali (crystalloid) in presence of the same percentage of serum protein or casein (colloid) increases with the original crystalloid concentration. This result is of fundamental importance because it disproves the universally accepted dictum that the colloid being a solid or liquid phase its concentration can be taken as constant. This point we raise at length further on, as it is at present entirely unappreciated, and shows an intrinsic difference between colloid and crystalloid. *Because the colloid concentration is not constant throughout the reaction, but varies like that of a gas or dissolved substance, the equilibrium equation does not reduce to one of the first degree, representing*

a reaction of the first order and with only one variable, viz., the concentration of the crystalloid; but remains an equation of the second degree with two variables, viz., concentration of crystalloid and concentration of colloid (see p. 67). The plotted points of the equilibria of a 'colloidal reaction' are hence roughly on an hyperbola and not on a straight line. They are only roughly so because the 'affinity constant' diminishes as more and more colloidal molecular combination between colloid and crystalloid goes on.

Another important point in which Mellanby is in accord in his results with Hardy and many other observers is that there is a *molecular* combination between salts and globulin. That the combination between a colloid and a crystalloid is one between molecules and not atoms is, in our view, the fundamental and distinguishing characteristic of a colloidal combination. The question is discussed at length by one of us in a paper now in the press,[1] and taken in conjunction with two other experimental findings this goes a long way towards explaining some of the seeming anomalies of colloidal compounds. The first of these two points is that what might be termed 'molecular valencies' can have much higher relative values than atomic valencies. Most atomic combinations giving origin to crystalloids take place with atoms having valencies not above four (as in carbon) or five (as in nitrogen); but in molecular unions or combinations one molecule may have a valency towards another as high as sixty to one, as, for example, in the case of our proteins with acid. We see for example a valency ratio represented by 24 to 1, when the alums combine with water to form the crystalline alums. For such molecular unions characteristic especially of colloids, there need be no complete atomic anchoring. A tendency to basic linkage of a weak type in a protein molecule, such as a feeble amido group, with no free atomicity serves to hold in 'molecular swinging' in the neighbourhood an acid *molecule* (not an hydrogen ion). There is an off and on kind of attachment, a dynamic rather than a static equilibrium, ready to vary up and down with varying conditions, to join more strongly or let go as slight alterations occur in the environing system.

This is the essential vitally important characteristic of the biotic molecule; and the simpler crystallo-colloidal compound, of alkali or acid or neutral salt with protein, leads up towards it.

In thinking, therefore, of crystallo-colloidal compounds, and still more so of living chemical units, we must depart from the statical, fixed, dead idea of a stereotyped molecule, and replace it by the conception of a

1. The rôle played by molecular affinities in bio-chemical reactions. B. Moore, *Archivio de fisiologia*, Vol. VIII, dedicato a Guilio Fano.

dynamical, labile, living molecule—variable and unstable in its component parts, and so giving a mechanism for energy exchanges, but as a whole stable and unaltering throughout a long series of subordinate interchanges.

When the concentration of the crystalloid reaches or exceeds that maximum at which the colloid is completely saturated, as occurs at the top of our series in each experiment, then the crystallo-colloid once more becomes fixed and like a stable purely crystalloidal compound. In such a condition it is useless in bio-chemical operations in the body, its essential lability is gone, and until crystalloidal pressure falls again it cannot react in the living cell.—Excess of oxygen acting upon living cells illustrates this point, and is as fatal to life as dearth of oxygen.

The number of researches upon the nature and composition of the union between casein and alkalies, and especially of casein and calcium, is quite extraordinary. Nearly all these confirm the facts first elucidated by Söldner, namely, that there are two classes of compounds of casein with bases: one neutral to phenol-phthalëin, termed the basic caseinate, and containing, according to Söldner, 2·32 per cent. of calcium oxide; the other neutral to litmus and formed when excess of casein is shaken up with dilute lime water or other alkali, the lime salt of which contains 1·55 per cent. of calcium oxide. As we have seen, the latter corresponds to our condition where no free alkali is present; the second, we have reason to believe, does not correspond to our maximum uptake of alkali, which corresponds very closely to double the amount for the neutral compound. Söldner's two figures are roughly in the ration of 2 : 3. Figures of different observers vary, but on the whole are in fairly close agreement for the so-called basic caseinates.

The caseinates of many bases have been prepared and investigated by W. A. Osborne,[1] such as ammonium, potassium, sodium, lithium, magnesium, strontium, and calcium caseinates. Osborne shows that casein acts as a stronger acid than carbonic acid, forming these salts from free casein and the carbonates or bicarbonates with expulsion of carbon dioxide, he also found that casein united with alkaloids such as caffein and strychnin. The caseinates of the alkaline earths form Ramsden surface films like milk on heating, and Osborne also found in confirmation of a forgotten discovery of Ringer[2] that the calcium caseinate undergoes a kind of coagulation on heating, which is reversible and re-dissolves on cooling. Para-caseinates are formed on acting upon the caseinates with

1. *Journ. of Physiology*, Vol. XXVII, p. 398, 1901; *ibid.*, Vol. XXXIV, p. 84, 1906.
2. Ringer, *Journ. of Physiology*, Vol. XI, p. 464, 1890; *ibid.*, Vol. XII, p. 164, 1891.

rennin, but as Ringer first showed, and Osborne confirms, are not thrown out until excess of calcium salt is present. Ringer was the first to elucidate the true action of calcium in milk-curdling.

The behaviour of casein with acids has not been quite so frequently investigated as in the case of the bases, but has received also a good deal of attention, as apparently being such a clear case of 'adsorption' on account of the insolubility of the casein-acid adsorpate.

Any account of the literature would be very incomplete without some reference to the most admirable paper by Lucius L. van Slyke and Donald D. van Slyke,[1] of the New York Agricultural Experiment Station, on the action of dilute acids upon casein when no soluble compounds are formed. The authors allowed carefully prepared pure casein to remain in concentrations of acids of 0·008 N and lower until equilibrium was established, filtered clear, and determined concentrations of acid in filtrate, from which amounts taken out by the casein could then be calculated by difference. The acid concentrations were determined in the filtrates by electrical conductivity and by titration methods. Over 0·008 N acid could not be employed because casein dissolved and interfered with the determinations. Conductivity water was used throughout, and long series of experiments carried out with hydrochloric, sulphuric, lactic and acetic acids.

The concentrations used by these authors were lower than our own, for we were able by the dialysis method to work without protein disturbance interfering with our titrations. Our results abundantly confirm those obtained by the van Slykes.

We completely agree with them that the process is one of adsorption in their experiments, and it is so in ours also, giving as it does all the criteria of van Bemmelen; we prefer, however, in spite of all this, to regard it (and all such adsorptions) as peculiar types of chemical reaction, which we should like to see called 'colloidal reactions.'

In the next section, in which we discuss our results in detail, we assign reasons why all components of the reacting system save the acid cannot be taken as constants, so that the reaction on a chemical basis does not for equilibrium reduce to the linear function $C_A = Kt$, where C_A is acid concentration, K a constant, and t temperature.

We are well aware of Ostwald's dictum that 'only gaseous and dissolved substances have varying concentration',[2] and agree that this holds

1. *Amer. Chem. Journ.*, Vol. XXXVII.

2. Ostwald, 'Principles of Inorganic Chemistry,' translation by Findlay, p. 327, 1902. Quoted by the van Slykes.

for crystalloids, such as the system [$CaCO_3$, CaO, CO_2] and many others. Here the constancy of concentration of $CaCO_3$ and CaO, the solid constituents, is obvious so long as there is any of either of them present; but is it so with a colloid? The colloid, as we have seen, is capable of uniting with 30 to 60 molecules of acid or alkali. Suppose at one stage each colloidal molecule is united with five molecules of acid or alkali, and at another stage is united with ten molecules of acid or alkali, and further suppose, as our experiments seem to indicate, that its 'affinity constant' decreases as it approaches complete saturation—is it then still to be regarded as of constant concentration? We believe it is then of varying concentration like any soluble constituent, and it is here that the intrinsic nature of the 'colloidal reaction' or 'adsorption' comes in. In our discussion we deduce the result, on this basis, that the curve should roughly be a hyperbola, and according to Ostwald's second additional criterion of adsorption quoted by the van Slykes the curve should approximate to an hyperbola.

On the exponential or logarithmic formula $\frac{C_1^k}{C_2} = K$ these authors find that the hydrochloric acid, lactic acid and acetic acid k is nearly 1, for sulphuric acid k is about 1·95 or almost 2. *Both* these sets could approximately lie on an hyperbola, the latter lying on the more curved part, and the former on the flat part where the hyperbola would almost appear as a straight line.

Whatever may be the interpretation of the results, experimentally they are in complete agreement—our own and those of the van Slykes—and, whether we ultimately find the problem is one of surface condensation and therefore so-called 'physical,' or one of specific colloidal reaction and therefore so-called 'chemical,' the value of the work as experimental research remains unaltered. At present, on account of the maximum value we have always obtained and because the action is a specific one for acids and bases with the proteins, we lean towards the chemical explanation.

Theoretical Discussion of the Results

The discussion of the meaning of our own results, especially in regard to the controversy as to chemical reaction *versus* adsorption, has been purposely postponed until after the statement of the experimental findings themselves, because we are of opinion that such results and the dialysis method for obtaining them in such colloidal solutions, possess a permanent

value as experimental matters, apart from any theory which may be given to account for them.

In the preceding critical account of the literature it has been impossible to avoid touching and commenting upon certain aspects of the contending theories; we desire now to deal more in detail with the theoretical side of the subject.

In regard to the nature of the action taking place, while it shows certain of the characteristics supposed to exist in typical adsorptions, and would be classed at present as a typical adsorption, it is in our opinion due to chemical affinity between the acid or alkali and the protein, and we would venture to suggest for all such reactions the name of 'colloidal reaction' instead of 'adsorption.' The word 'adsorption' is an essential one to retain, but it ought not in our opinion to be used to cover sets of phenomena so different as, condensation of moisture on glass; absorption of gases by charcoal, kaolin, and other inert bodies; uptake of water by fibres; union of dyes with fibres and tissues; formation of surface films on liquid and solid interfaces of a two-phase or three-phase system; unions of proteins or other colloids with acid, alkalies, and other electrolytes; and formation by molecular union or complex formation of inorganic colloids, such as the sulphides, etc.

There exists no experimental evidence that all these divers types of interaction are identical in kind and in causation, and the use of the single word 'adsorption,' to signify them all, is highly unscientific and misleading, and calculated more to cloak ignorance than to lead to experimental investigation.

Some of the above phenomena are almost certainly purely physical and due to surface cohesion, where molecular affinities enter in, not at all, or in very diminished degree; others are partly physical and partly chemical,[1] that is to say, dependent on molecular relationships arising from chemical dissimilarity, and we feel certain that some of them, such as those we are dealing with here, are almost purely chemical. If the word adsorption could be restricted to signify the purely physical process or the physical portion of a mixed process, and the term 'colloidal reaction' be applied to the chemical process where a colloid takes part in a reaction, or to that portion of the mixed process (such as dyeing of fibres

1. We dislike to use the word 'chemical' in contradistinction to 'physical,' as in the last resort both chemical and physical phenomena are ultimately physical in the broad sense of the word. We use the two words in obedience to established usage, meaning by 'physical' energy forms not involving *specific* molecular energies, and by 'chemical' energy exchanges which occur specifically between two chemically dissimilar substances, causing them to become more firmly united as a result of their specific chemical dissimilarity.

probably is) which is chemical, we believe a distinct advance in terminology of colloidal unions would be made.

Before passing to the more detailed consideration of the interpretation of our own results, we would also enter a protest against the scientific absurdity of allowing one single characteristic and that often of a doubtful nature, to outweigh all other evidence, no matter how strong or based on a number of different well-established facts.

No result bearing upon these colloidal unions seems nowadays to be of any value unless it can be plotted in a curve. Men appear only to be able to think in curves, although figures are often quite as easily understood, and show results better and more truthfully to those who will take the pains to examine them. There is nothing so easy as by proper (or improper) use of suitable co-ordinates, and choice of different scales of magnification in plotting ordinates and abscissae, to gloss over an imperfectly carried out experiment, and by judicious use of French curves make the experiment illustrate either one formula or another quite different one. Certain parts of a hyperbola or parabola can be made to look very like a straight line, or by the judicious choice of (k) a logarithmic curve can have for a good way an indistinguishable look from a conic, and lie so close to it, that a set of experimental points might well belong to either curve. After the curve has been plotted, the next thing is to see whether a presentable number of the experimental points can be tortured into, or somewhere near to, a curve represented by the formula $\frac{C_1^k}{C_2} = K$, and if this can be done, no matter how near k may lie to the values of 1 or 2 then, there is no help for it, and the case is one of adsorption.

This is really no exaggeration of the view taken by some recent authors, who appear to close their eyes, under the hypnotic influence of this equation and its supposed curve, to all opposed experimental facts.

For example, the union of oxygen with haemoglobin, a subject of fundamental importance to be correctly understood in bio-chemistry, has for years been believed to be a dissociable chemical union. The evidence in regard to this comes from several discrete sources, and appears to be about as well an established fact as possible in chemical science.

For once, thanks to the iron which the haemoglobin molecule contains, its molecular weight, although it is colloidal, is well known; it can be obtained in pure crystalline form and analysed, and its molecular weight can also be determined by direct measurement of osmotic pressures. The amount of oxygen it takes up (although it varies with the oxygen

pressure at low pressures) at and above the partial pressure of oxygen in the earth's atmosphere, is practically constant. Hence the amount of oxygen capable of combining at saturation point with a known weight of haemoglobin is well and definitely known. Further, the stochiometric relationships, on comparing the two combining weights, are of the simplest description, viz., one molecule of haemoglobin with one molecule of oxygen.

This would appear to be one fairly strong piece of evidence that haemoglobin and oxygen enter into chemical union, and that the smaller amounts of oxygen at lower pressures are due to partial dissociation.

Further, haemoglobin unites with other gases, and its union with carbon monoxide has been particularly well worked out. Here the coefficient of the reaction is higher, and saturation occurs at a lower partial pressure with carbon-monoxide than in the case of oxygen. But, at the saturation point, carbon-monoxide replaces oxygen, volume for volume, and the stochiometric relationships are again one molecule of haemoglobin to one molecule of carbon-monoxide. This again is somewhat convincing as to the action being of a chemical nature.

Lastly, if we look at the spectrum of haemoglobin free of oxygen we see a quite characteristic definite spectrum, with one well-marked band; allow access of oxygen, and the spectrum at once changes to a quite different, but equally characteristic, two-banded one, which reverts again to the one-banded spectrum if the oxygen be pumped off. At the same time that these spectroscopic changes occur the solution markedly changes in colour, showing a dark purple when free of oxygen, and a bright scarlet when united to oxygen. These are the chief points of evidence in favour of the existence of a chemical union between haemoglobin and oxygen, and, to our minds, they are quite convincing.

But simply because the points showing amounts of oxygen taken up at various partial pressures *appear* to lie on an exponential curve, all this evidence has been neglected by a recent worker on colloids, and the phenomenon of oxygen and haemoglobin union described as an adsorption.

It seems to be pretty unanimously forgotten by workers on the subject that an exponential curve is the very prototype of an *empirical* curve, the formula as the exponential index is altered, giving a series of traces like a set of French curves, to one of which nearly any set of experimental points can be made to approximate with that degree of accuracy usually expected in such a case.

Another assumption, experimentally unwarrantable, which has been

made in many papers devoted to such discussions on adsorption or chemical reaction is, that, supposing the union to be a chemical one, the ratios of free and combined crystalloid must be very simple because the concentration of the colloid does not alter during the reaction. The reason assigned for the colloid concentration not altering is that it is not in true solution but exists as a solid or fluid phase, in a heterogeneous system with interfaces between its aggregate and the solvent, which contains the crystalloid in varying concentration. It is in fact exactly similar to a plate of a metal, such as zinc, being attacked by an acid, and as long as there remains excess of metal its concentration (which is merely represented by its surface) may be taken as constant and written down as such in the equilibrium equation.

Unfortunately for the study of colloidal changes with crystalloids, the above reasoning is entirely fallacious; the colloid cannot be regarded as a discrete phase of solid, and therefore possessing a constant concentration, until it is all used up. Its concentration varies throughout the reaction just as does that of the crystalloid uniting with it, and it is this fact which makes all the difference to the form of the equilibrium equation, on the provisional assumption of chemical combination. Even we assume that there are interfaces between the colloidal aggregates and the solvent, large compared to the molecular dimensions, so that the colloidal solution comes to resemble an ultra-microscopic emulsion. Still, we have no grounds for assuming that the concentration, being represented by united superficial area of the aggregates, is constant, and hence that crystalloidal pressure (acid or alkali concentration, for example, in our experiments) must remain nearly constant and at a low value until all this surface is covered with adsorbed material. For, as crystalloid is attracted to the colloidal surface, the attached crystalloidal molecules will all the time be packed closer and closer together, and this can only be accomplished by a higher and higher concentration of crystalloid in the solvent. Whether, therefore, we take a physical or a chemical basis to go upon, the concentration of colloid, as measured by its surface or otherwise, does not remain constant, but continuously diminishes just as if it entered into true chemical union. Accordingly we have no right on the basis of chemical union to expect that great simplification of formula and form of curve which would arise if the colloid concentration were constant. It is necessary to point this out at length, because this erroneous assumption has been made by previous authors and has led to the wrong criterion, that in case there is chemical combination, then the amount of crystalloid

taken up at varying concentrations of crystalloid must remain constant, which it does not do experimentally. Also, it has been supposed that with less crystalloid present than that required for union with the whole of the colloid, then all of the crystalloid should be taken up.

Again, in the case of union of a mono-molecular type, where one molecule of colloid unites with one molecule of crystalloid, as we have seen is the case with haemoglobin and oxygen, investigation of the equilibrium equation shows that instead of the relationship being a simple linear one between oxygen absorbed and partial oxygen pressure, as it would be if the concentration of the haemoglobin remained constant, it becomes a conic section oblique to the axes of co-ordinates. An investigation of the equilibrium equation is mathematically exceedingly simple, and as it well illustrates the point at issue, we venture to give it.

The identical reasoning employed will hold for any case in which one molecule of a colloid unites chemically with one molecule of a crystalloid,[1] but as the form of the oxygen absorption curve with varying oxygen partial pressures is familiar to all biological chemists, we shall describe the deduction of the formula in terms of oxygen and haemoglobin.

Let C_1 = concentration of oxy-haemoglobin.
,, C_2 = ,, of reduced haemoglobin.
,, C_3 = ,, of oxygen in oxy-haemoglobin (i.e. absorbed oxygen, usually plotted as one co-ordinate by Hüfner or Bohr).
,, C_4 = ,, of free oxygen (proportional to oxygen partial pressure and usually plotted as the other co-ordinate).

This means that C_1, C_2, C_3, and C_4 are the four concentrations, which exist together at an equilibrium point for any given partial pressure of free oxygen (C_4).

Then, we have first the following facts to simplify our equation:— Since the total amount of haemoglobin present either as oxy- or reduced haemoglobin is constant, it follows that oxy-haemoglobin *plus* reduced haemoglobin is a constant, say A, that is

$$C_1 + C_2 = A$$

Next, since we have as an experimental fact that one molecule of haemoglobin unites with one molecule of oxygen, it follows that the concentrations of oxy-haemoglobin and of absorbed oxygen in it are equal, or, in other words, $C_1 = C_3$.

1. The same equation as we shall show later also holds for a multimolecular reaction between a colloid and crystalloid, unlike the case for two crystalloids of different valency uniting.

Coming now to the equilbrium equation, the tendency to union of reduced haemoglobin and the free oxygen is proportional to the concentrations of these two substances, i.e., to $C_2 C_4$, and the tendency to move in the other direction by dissociation of combined oxygen from combined haemoglobin is proportional to the concentration of these two, i.e., to $C_1 C_3$, or since $C_1 = C_3$ to C_3^2. Accordingly at the equilibrium point, since these two tendencies balance, we can introduce a constant and equate them, giving us, as equilibrium equation:—

$$C_2 C_4 = k C_3^2$$

To get an equation between the two amounts of free oxygen (C_4) and combined oxygen (C_3) we must eliminate C_2 (the concentration of reduced haemoglobin). This elimination, the equation $C_1 + C_2 = A$, enables us to perform for $C_1 = C_3$ $\therefore$ $C_3 + C_2 = A$ or $C_2 = A - C_3$. Substituting this value for C_2 we get finally for our equilibrium equation between C_3 and C_4:—

$$(A - C_3)\ C_4 = k C_3^2, \text{ this may be written}$$

$$k C_3^2 + C_3 C_4 - A C_4 = 0, \text{ where } C_3 \text{ and } C_4 \text{ are two variables.}$$

This final form is a conic with its axes oblique to the axes of co-ordinates, and may be a parabola or hyperbola, according to the experimental value of k. Anyone familiar with the dissociation curves of Hüfner and Bohr will realise how closely they resemble hyperbolae or parabolae.

This is quite a different result from the straight line or linear relationship of direct proportionality between oxygen taken up and partial oxygen pressure, which follcws if oxy-haemoglobin concentration is supposed to remain constant, and so both theoretically and experimentally the assumption of constancy of concentration of colloid in such interactions is shown to be erroneous.

There is a most important and fundamental difference between the crystalloidal and colloidal chemical reaction, to which no attention, to our knowledge, has hitherto been given. It may be formally stated thus:— As the valency of one of the substances increases in a purely crystalloidal reaction, the degree of the equation of equilibrium goes up accordingly, and the conditions of equilibrium become excessively complicated; on the other hand, in an equilibrium where one of the constituents is a colloid, although the colloid molecule or solution aggregate may be capable of combining with a huge number of crystalloidal molecules (such as thirty to sixty, as in our experiments in this paper, see page 43), the degree of the equilibrium equation does not rise, but it always remains roughly expressed by an equation of the second degree.

In the reactions of serum proteins with alkali and acid, which we have been experimentally examining above, there is clear evidence that each solution aggregate of protein is capable at the saturation point of union with thirty to sixty molecules of alkali or acid. One almost shudders to think of the complexity of the equilibrium equation deduced on the basis of chemical combination on the usual lines for such a reaction; it would certainly be an equation of high degree, and require many experimental points for the solution of its constants.

Does this necessarily rule out chemical combination altogether? By no means; we have simply been translating our preconceived notions, founded on crystalloidal reactions to a region where they do not hold, and where the phenomenon though still chemical (or possibly chemical) is arranged differently.

The obvious difference which alters the whole situation is that intermediate compounds with variable numbers of attached crystalloids can exist in the case of the colloid and that these cannot exist in the case of the two crystalloids of different valency combining with each other to form a crystalloidal compound. Suppose A, for example, is a trivalent crystalloidal ion and B a monovalent ion, capable of uniting to form the crystalloidal compound AB_3, then for the formation of a molecule of AB_3, the simultaneous collision of three molecules of B upon A is required (using the kinetic theory to state our case). This means (if C_A and C_B express concentrations of A and B respectively) that the tendency towards union is measured by the product $C_A \cdot C_B^3$, and the opposite tendency to dissociation or ionization is expressed (C_{AB_3}, being the concentration of the compound AB_3) by C_{AB_3}. Accordingly, the equilibrium equation becomes $C_A \cdot C_B^3 = k \cdot C_{AB_3}$, which works out to an equation of the fourth degree. If there were any cases of very high valencies, such as thirty or sixty, amongst crystalloids we should have equilibrium equations of similarly high complexity.

Now, all this follows because such compounds as $=AB''$, or $-AB'_2$, cannot exist in the case of crystalloids. For if they could exist there would be no need for three ions of B to be within the sphere of influence of A at the same instant. The intermediate ion ($-AB''-$) could remain stable and in a certain concentration in solution, till it was joined by a second B, and became $-AB'_2$. Similarly, $-AB'_2$ could exist until it was joined by a third B′ and become AB_3, after which no more of B′ could be taken up. This last stage is precisely what has happened in our present experiments when the amount of 'united' acid has reached its fixed maximum point.

It is this difference in behaviour of colloidal reactions which distinguishes them from incomplete mono- or bi-molecular reactions of crystalloids. The laws governing an adsorption, or, as we prefer to call it, a 'colloidal reaction,' differ in certain fundamental respects from those governing an incompleted or balanced crystalloidal reaction, and hence, *even on the view that both are purely chemical*, the one is different from the other.

One fundamental difference we have just pointed out, namely, that no matter that even thirty or sixty crystalloidal molecules or ions are uniting with one solution aggeggate of colloid, the degree of the equation does not rise but is *roughly* represented by an equation of the second degree. Simply because any number from one to one hundred of the crystalloidal molecules for which attachments are possible may be present on an aggregate at any given time, or the attachment may be vacant. Another important difference is that the colloidal is a reaction between molecules, the incompleted crystalloidal reaction is one between atoms or unsaturated radicles.

We may now further enquire why the second degree equation represents equilibrium conditions in the colloidal reaction, *so much more roughly*, than it does the simple reaction between two crystalloidal equivalent ions or molecules.

To understand this we must return to the derivation of the equation of equilibrium and the assumptions tacitly made therein. The equation of equilibrium for two crystalloids tending to unite is derived by assuming that the tendency to union is determined *solely* by the concentrations at the moment in the solution of the two ions or molecules uniting. If the two concentrations are C_1 and C_2 and we introduce a constant k_1, we can express this by equating it to $k_1 C_1 C_2$. On the other side the tendency to disrupt is proportional to the concentration of the substance formed (C_3), and again introducing a constant this is measured by $k_2 C_3$. For equilibrium, the two tendencies balance, and we get the usual equilibrium equation $k_1C_1C_2=k_2C_3$. Now, all this holds rigorously enough for the crystalloidal reaction where there are no intermediate stages or any gradual accumulation of the one combining substance on the surface of the other. Hence the constant is here a real fixed constant, the tendency to union being determined *solely* by solution concentrations. With the colloidal reaction, however, the case is different, and something more than concentrations comes in. For, as the concentration of crystalloid goes on

increasing, the colloidal aggregate becomes more and more charged up with crystalloidal ions or molecules already in place. The presence of such united ions must obviously have a *secondary* influence on uptake of further crystalloidal ions or molecules, *in addition simply to the increased number of free places*, the change in which number alone is expressed by the changing concentration. The action is somewhat similar to that seen in constructive synthesis in organic chemistry, where each successive similar group or radicle is only introduced into a given molecular conformation with increasing difficulty, although a similar place or linkage exists for it. This means that k will not remain constant, but, *for these secondary causes*, will vary as the concentration of the crystalloid undergoes considerable variation.

On exactly similar lines to those given previously for haemoglobin and oxygen (see p. 68) an equation can be deduced to represent the equilibrium between the serum proteins and acid or alkali concentrations of the form $k C_s^z + C_1 C_2 - A C_2 = 0$, where C_1 and C_2 are concentrations of united and of free acid respectively, and A is the total available combining power of the protein. But, for the above reason of decreasing affinity between partially combined aggregates and further crystalloidal acid or alkali, it is obvious that k will continuously decrease as C_1 approaches A in value; that is to say, as complete saturation of the protein is approached. This is precisely what happens when such an equation derived on the assumption of a constant specific affinity (the chemical affinity is represented by the constant k) is applied to an experimental case, where the affinity is all the time diminishing as the concentration of the acid or alkali is forced up.

In the limiting case, where the concentration of the combined acid or alkali (C_1) becomes equal to A; that is to say, when complete saturation occurs, the second and third terms of the equation balance, and we get $k C_1^z = 0$, and since C_1 is not zero k must now have reached a zero value. This means that at the saturation point, all chemical affinity for crystalloid has been satisfied.

Substitution of the values for C_1 and C_2 from our tables in the experimental part of the paper shows the same result that k is decreasing with each increase in C_1 and C_2, or, in other words, the affinity is growing less continuously.

By empirically employing an exponential (or logarithmic) equation of the form $C_1^k = KC_2$, values for k can be obtained which, instead of varying continuously in one direction, will wriggle round a mean point with irregular variations accompanying similar perturbations in the experimental technique. But it is absurd to attach any importance to a constant so obtained. We do not know what it means. It certainly is

not an affinity constant, nor one related to any known physical or chemical property, but purely and simply an empirical figure. By so doing we are merely grinding out of a mathematical equation an unknown result, which we have thrown in by making an unknown assumption. We should simply be satisfying our minds unwarrantably, by obtaining a constant of the meaning of which we possessed no knowledge.

The value that we get from the simply deduced equation from the mass action law is, first that it demonstrates to us that there is an upper limit of possible union of the acid or alkali with the protein or other colloid. This is also demonstrated by our experiments. Secondly, both equation and experiments show that free colloid and free acid or alkali exist together, and a certain value of concentration is necessary for a definite amount of combination. Thirdly, we see that the *affinity constant* diminishes with increasing saturation, and becomes zero at complete saturation. Inspection of the figures also shows this decreasing affinity. Lastly, considering the results in conjunction with the known osmotic pressures of the serum proteins, we see that a large number of acid or alkali molecules or ions unite with each solution aggregate of protein, and the form of the experimental results demonstrates that this occurs in such a way as to distinguish completely the colloidal reaction from the incomplete crystalloidal reaction. The difference lies in the colloidal reaction proceeding by stages, while in the crystalloidal reaction of higher degree this is impossible, the intermediate stages being incapable of existing.[1]

We venture, in conclusion, to make a final protest against a loose form of expression which has crept into the literature of colloids in describing adsorptions (or colloidal reactions), namely, that they do not obey the mass action law. This is used, quite wrongly in our opinion, as a great criterion of physical adsorption *v.* chemical combination. Narrowly examined it really is found to mean, that the phenomenon, *as postulated and understood by the authors*, does not obey the law of mass action.

The reason why it does not *appear* to obey the mass action law is that there are secondary and varying factors at work which have been summarily left out of the equation. We get out of mathematics what we have put in, ground round for us into another shape.[2] That is all.

1. This assumption is quite different from that of a long series of intermediate bodies described earlier in the paper; for there is but one body, the colloid, at various stages of saturation with a second body, the crystalloid; in the so-called ion-protein theory, there are a vast number of intermediate saturated complexes or 'ampholates,' each forming a chemical individual.

2. Mathematics may be compared to a mill of exquisite workmanship, which grinds you stuff in any degree of fineness, but, nevertheless, what you get out depends on what you put in, and as the grandest mill in the world will not extract wheat-flour from peascods, so pages of formula will not get a definite result out of loose data. (Huxley, 'Lay Sermons,' p. 216.)

Substances capable of reacting upon one another can no more disobey the mass law than an energy-change can disobey the fundamental laws of thermo-dynamics, or a falling body disobey the laws of gravitation.

A feather floating in the air *appears* to be disobeying the law of gravitation until we understand the phenomenon, and then the disobedience disappears.

In our view, then, such unions as we have been studying are chemical, in that they occur between bodies still of molecular dimensions; in that the attraction between such bodies is of a specific character and dependent on the molecular conformation of the two reacting bodies; in that (especially at the upper limit) definite mass relationships (or stochiometric relationships) exist; in that molecular valencies occur, although the figures representing ratios of valency are higher than in the case of atomic reactions.

But we are well aware that such unions, because they occur between molecules and not between atoms, are a step onwards towards the more general cohesions and surface reactions which may be called 'adsorptions,' and are generally regarded as purely physical in character.

There is no real demarcation between physical and chemical phenomena. In modern physico-chemical notions, the atom is losing its position as a fixed constant, just as is that of the cell in cytology and biochemistry.

Just as we have now to concede ultra-microscopic organisms and invisible germs, and have to take the enzyme as something endowed with energetic properties from the cell, a kind of bio-chemical unit of lower value than the cell; so in physics we are coming to regard the electron as a more minute form of matter than the atom, a something capable of carrying an electric charge, which, added to or taken away from the atom, changes its character. Proceeding in the opposite direction, when we pass one stage upward from atomic reaction we come to molecular reactions, or, as we have termed them, colloidal reactions; thence to cell formation. There is nowhere any hiatus but only a series of gradual transitions. It is at these transition points that interesting problems await investigation. It is also just at these interesting points that cause and effect are so difficult to correlate. For example, where two phases exist with a contact surface, it can be demonstrated thermodynamically that if the presence on the surface layer of any dissolved substance tends to lower surface-tension then that substance will increase in concentration on the surface. Is this a physical or a chemical

result, and which is cause and which effect? At first sight it looks as if it were a purely physical change and that there is no specific molecular affinity, or, in other words, no chemical reaction. But why does the dissolved substance lower surface tension? There must obviously be something specific in this because it does not occur markedly with every solvent and solute, and in cases chosen specifically for examination by experiment it is quite obvious that there is specific affinity between solvent and solute. Even in ionized aqueous saline solutions, the older simpler view of perfectly free ions in solution, having served its day is passing away on account of the many things it quite fails to render any account of, and it has to be recognised that there are complexes (or molecular unions) between ions and solvents. These complexes on the one hand are not the old stereotyped solution hydrates, but on the other they remove for ever the free unchained ion, and introduce the conception of a chemical union between ion and solvent, which union is initially responsible for the ionization.

So, too, in the case of surface tension and surface film formation, the physical changes are dependent upon chemical affinities. The surface tension is lowered because the dissolved colloid and its solute are tending to unite in molecular union; on or near the surface, the dissolved colloid is less under the disruptive influence of the solvent acting in mass and tending to send it into solution. A colloidal particle arriving on the surface has passed half way out of solution, and if there is anything on the surface with which it can pass into molecular union or adsorption it will remain bound there. If, however, there is nothing with which it can unite on the surface, it must raise the surface tension, because it remains there in true solution, and all molecules in true solution must raise surface tension, because surface tension is in two dimensions what osmotic pressure is in three dimensions. This is obvious, because if we dissolve a substance in true solution in a solvent the osmotic pressure shows the volume energy has increased. If now we take equal volumes of solution and of pure solvent and draw these out into two equal and similar surface films, so thin that the whole thickness of the layer of each is within surface action, then it follows that the difference in amount of work necessary to do this must exactly equal the work of solution, for now surface tension replaces the properties of volume, and all the previous volume energy must be located on the surface.

To return to surface film formation then, if the solute remains in true solution on reaching the surface it must raise surface tension, and

hence an equilibrium must soon be established. No surface film will be formed under such conditions, and there can be no adsorption. On the other hand, if the molecule reaching the surface enters into molecular union either with similar molecules or with dissimilar molecules for which it possesses molecular affinities lying on the surface or interface, then surface tension will not rise, and surface films will form or surface condensation occur.

Without some such *specific* factor—which we may term physical or chemical, as we choose—there is no obvious reason why adsorption coefficients should vary in each individual case. Why, for example, palladium should not adsorb all the permanent gases equally, or haemoglobin take up nitrogen, oxygen, and carbon monoxide in equal amount at equal pressures.

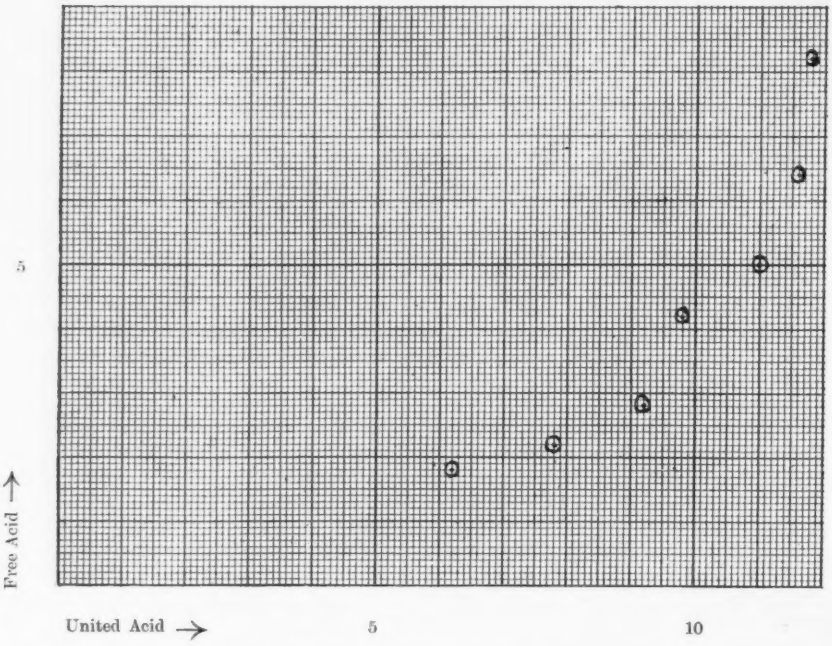

FIG. 1. *Expt. XIII.*—EQUILIBRIUM OF SERUM PROTEINS WITH SULPHURIC ACID.

Ordinates show free acid as 1 1000 N, and abscissae united acid in c.c. of N/10 to 0·703 grm. of serum protein in 7·03 per cent. solution.

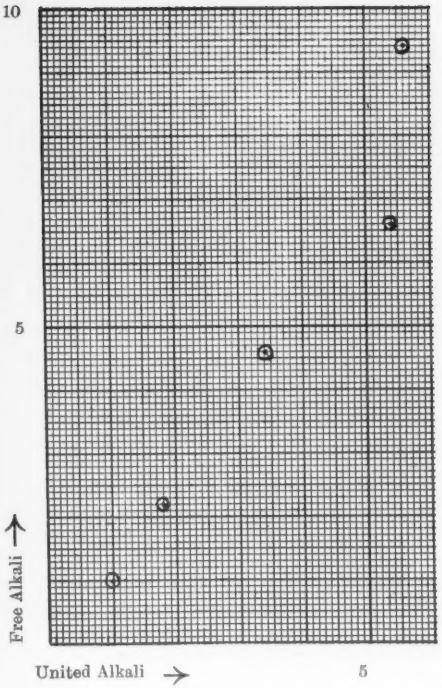

FIG. 2. *Expt. XVI.*—EQUILIBRIUM OF SERUM PROTEIN WITH ALKALI (NaOH)

Ordinates show concentration of free alkali in 1/1000 N, abscissae united alkali in c.c. of N/10 alkali uniting with 0·687 gramme of protein in 6·87 per cent. solution.

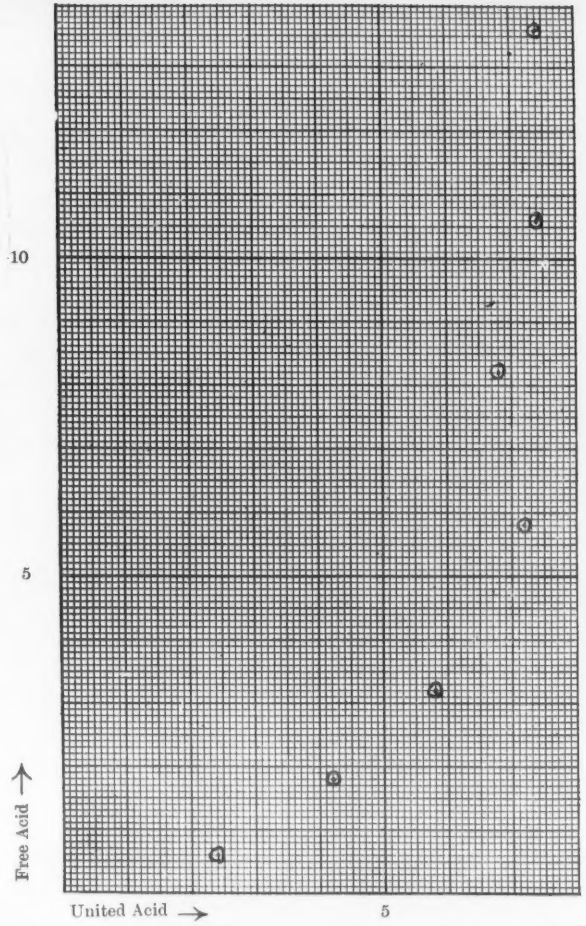

FIG. 3. *Expt. XVII.*—EQUILIBRIUM BETWEEN CASEIN IN 9 PER CENT. EMULSION AND SULPHURIC ACID

Ordinates show concentration of free acid in N/1000; abscissae amount in c.c. of N/10 acid taken up by 0·9 gramme of casein in 9 per cent. suspension.

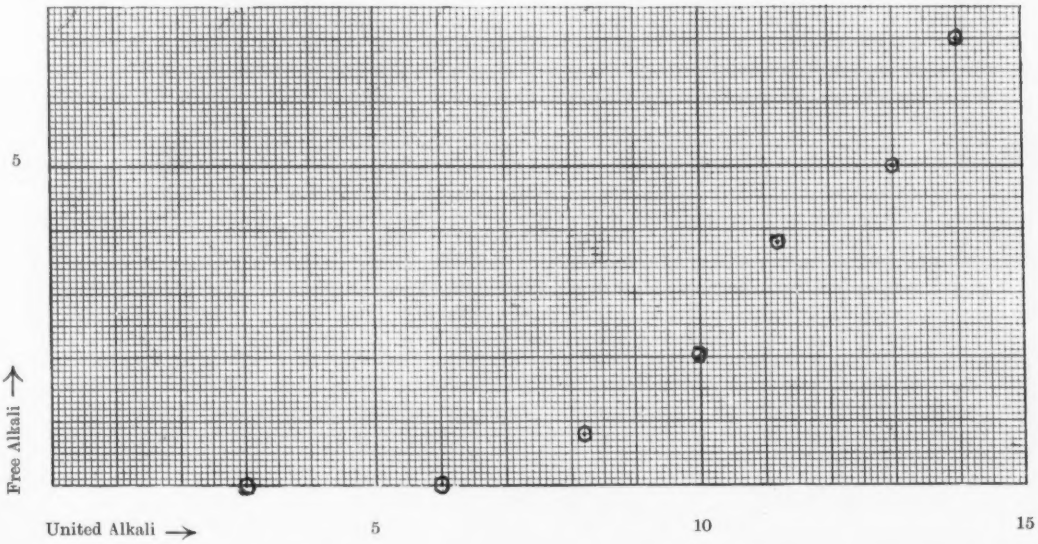

FIG. 4. *Expt. XVIII.*—EQUILIBRIUM BETWEEN CASEIN IN 9 PER CENT. CONCENTRATION AND ALKALI (NaOH).

Ordinates show free alkali in N/1000 concentrations, abscissae united alkali in c.c. of N/10 united with 0·9 gramme in 9 per cent. concentration.

THE ACTION OF ETHER UPON THE CIRCULATION

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(*Received December 2nd, 1909*)

Ether has, in comparison with chloroform, received little attention from pharmacologists; in the few investigations of its action that have been carried out, the ether research has usually been a small appendage to a research on chloroform. Although Snow and Ringer long ago showed experimentally the many advantages as an anaesthetic that ether held over chloroform, the present extensive employment of ether is mainly based on clinical experience. As long ago as 1848, Snow (1) found experimentally that ether produced more efficient anaesthesia than a quantity of chloroform which produced an equal degree of narcosis, and that chloroform was eight to ten times more toxic than ether.

Ringer (2) showed that the isolated heart was accelerated by a small percentage of ether in the circulating fluid, but that large percentages arrested the heart in diastole. To produce this effect upon the heart, fifty times as much ether as chloroform was necessary. The Glasgow Committee (3) found that ether, when given in quantities just sufficient to induce anaesthesia, had but little effect on blood pressure or respiration. Wood (4) found that ether in moderate doses acted as a stimulant to the circulation, but that in very large doses it was capable of depressing the heart, though the cardiac depression was usually less than that of respiration. He mentions two experiments, however, in which death occurred from syncope, respiration continuing for one or two minutes after cessation of the circulation.

McWilliam (5) found that ether in anaesthetic quantities produced no cardiac dilatation but that with large doses this sometimes occurred. He concluded that the fall of blood-pressure usually observed was mainly due to vaso-dilatation, secondarily to which followed diminution in the force of the heart beat.

The Hyderabad Commission (6) reported that ether produced the same bad effects as chloroform; when given by the open method it was almost as dangerous, when given by a closed inhaler it was quite as dangerous, as chloroform. Cushny (7) found that pure ether vapour produced respiratory paralysis plus cardiac paralysis in the same way as chloroform; he concluded that the difference between the effects of chloroform and ether was mainly one of degree.

Other observers found ether to be in every respect less dangerous than chloroform.

In the present research, first the general effects of ether upon the intact animal were observed, both the open method of administration, i.e., without restriction of air, and the closed method, i.e., with restriction of air, being employed. Then the action of ether upon those organs likely to contribute to the general effects was investigated, the isolated heart, the vagus mechanism, the vaso-motor mechanism and the respiratory system being in turn subjected to the action of the drug. Dogs were employed throughout the investigation.

The Effect of Ether upon the Intact Animal

Ether was first administered with a free supply of air. The higher the percentages of ether, the more rapidly was anaesthesia induced. In all cases where a percentage of ether vapour sufficient to induce anaesthesia was administered, the blood-pressure fell, the rate of fall depending upon the strength of the ether vapour. This was the case with either natural or artificial respiration.[1]

Table I

Effect upon blood-pressure of varying precentages of ether vapour in air respired:—

Percentage ether	Period of administration	Blood-pressure at commencement	Blood-pressure at end
10·5	31 min. 20 sec.	104 mm. Hg	39 mm. Hg
14·3	15 „ 10 „	98 „	50 „
20·0	5 „ 10 „	90 „	46 „
31·5	8 „ 20 „	92 „	0 „

This fall of pressure is illustrated in Fig. 1, which was obtained during an experiment in which a high percentage of ether was administered by natural respiration. In ten minutes the blood-pressure fell from 102 mm. Hg to zero. In experiments in which a slight degree of asphyxia was maintained throughout the administration—thus imitating ordinary clinical conditions—the blood-pressure always rose, the extent of the rise depending on the degree of asphyxia. Even when ether was now administered by the open method, the blood-pressure remained for a considerable time at a higher level than the normal.

Fig. 2 illustrates an experiment in which ether was given by a Clover's inhaler, the cylinder of which was attached directly to the

1. The percentage of ether vapour was determined at room temperature by means of an oil absorptiometer.

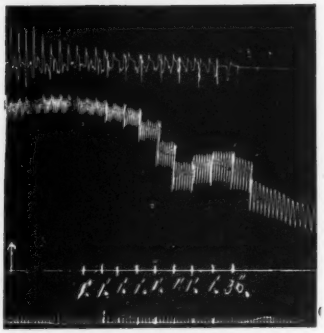

FIG. 1.—Effect of Ether in high percentage administered by open method to intact animal. Fall of blood pressure, respiratory failure, final heart failure from lack of oxygen.

[NOTE.—The scale of reproduction for all the tracings in this paper is one-half linear.]

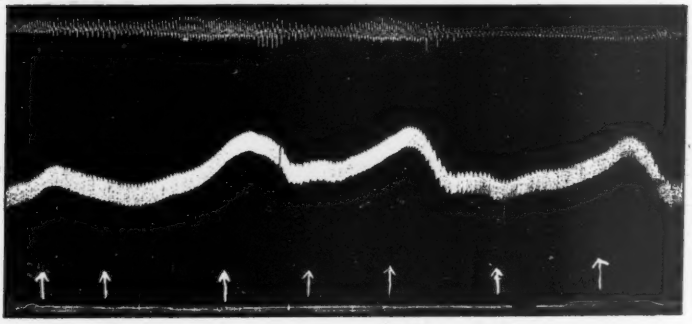

FIG. 2.—Effect of administration of Ether with limitation of air supply. Administration had been in progress 2 minutes before commencement of tracing. Blood pressure 110 mm. at start, 116 mm. at beginning of tracing. Administration of Ether by open method 2 minutes after conclusion of tracing caused a fall of 10 mm. Hg in 6 minutes.

tracheal cannula. On commencing the administration the blood-pressure was 110 mm. Hg, during administration it rose to 150 mm., after the administration it remained for some minutes about 120 mm. When it had fallen nearly to its original level, ether was administered by the open method. In six minutes the blood-pressure fell only 10 mm., in marked contrast to the result usually produced by the open method.

In all experiments in which ether was given by Clover's inhaler there was a rise of blood-pressure. The main factors in this rise were probably vaso-constriction, augmentation of cardiac contraction, and mechanical effect on the circulation of increased respiratory action, all three being due to asphyxial stimulation.

In these experiments the effect of ether upon the heart beat varied with concentration. With 10 per cent. of vapour the heart was quickened, with 15 per cent. it was unaffected, with 20 per cent. and upwards it was always slowed.

The Effect of Ether upon the Heart Isolated from the Central Nervous System

The few previous observations on this point show considerably diverse results.

Ringer (2) found that the isolated frog's heart was accelerated by a small amount of ether in the circulating fluid, but that large percentages arrested the heart in diastole. To produce this effect, he found fifty times as much ether as chloroform was necessary.

Bock (8) made observations on the isolated dog's heart, using a heart and lung circulation. He found little or no fall of blood-pressure in the circulation, although inhalation of ether was continued for a long time. If the blood-pressure did fall, it rose after cessation of administration higher than at the beginning of the experiment. The heart remained regular and unaltered in frequency.

Dieballa (9), using Williams' apparatus, perfused excised frogs' hearts with 'isotonic and isoviscous' solutions containing small proportions of ether. He found solutions containing 0·25 per cent. ether to be without influence on either force or frequency. With solutions of greater strength the heart's contractions became weaker and irregular, and with solutions of 2·5 per cent. to 3 per cent. diastolic standstill came on in less than a minute. The heart soon recovered on perfusion with isotonic saline.

Derouaux (10) found that the cat's heart, perfused with Locke's fluid containing 0·15 per cent. ether, soon stopped, but rapidly recovered when perfused with Locke's solution; the dog's heart became weaker and slower in beat with 0·25 per cent. ether.

In all the present observations, evidence of depression of the heart's action was found. The method employed was a modification of that used in a former research on ethyl chloride (13). The cardiac nerves were all divided. The aorta was ligated beyond the origin of the left common carotid. All the arterial trunks springing from the arch of the aorta were then ligated, with the exception of the innominate and the left common carotid. The right subclavian was ligated, and the right carotid was connected with a blood-pressure recording apparatus. The left external carotid was then ligated so that the only arterial path left open was the left internal carotid. This artery was substituted for the brachial which I employed in former researches, because under the action of ether the latter artery undergoes marked relaxation. Such relaxation does not take place in the internal carotid, enclosed as it is in the unyielding skull. The blood, returning to the heart by the jugular veins, passed through the right heart and pulmonary circulation, where it was aerated by means of artificially ventilated lungs, thence to the left heart.

In eighteen experiments on the 'isolated' heart, the degree of depression produced increased with the strength of ether vapour administered. Since, however, even with the same percentage of ether, the depression produced in different hearts varied considerably, it is impossible to institute strict comparisons. Some results approximating to the average are shown in Figs. 3, 4 and 5, from which the following figures are calculated.

TABLE II

Effect of increasing percentages of ether in inspired air on 'isolated' heart:—

	Percentage ether	Duration of administration	Blood-pressure at beginning	Blood-pressure at end
Fig. 3	10·5	27 min.	180 mm. Hg	140 mm. Hg
Fig. 4	19·5	2 „ 20 sec.	186 „	76 „
Fig. 5	30·2	1 „ 30 „	200 „	68 „

Comparisons of the fall of blood-pressure that occurs in a given time in different experiments with different percentages of ether show that the increase of toxicity is not proportional to the increase in strength of vapour.

From a comparison of results obtained in these experiments and some obtained in experiments on the action of chloroform, I have calculated that 30 per cent. of ether vapour produces effects of the same order as 1 per cent. of chloroform vapour. For example, in an experiment on the action of chloroform on the 'isolated' heart (Fig. 1 in chloroform paper) (12), 1·2 per cent. chloroform vapour in the air inspired brought the blood pressure from 200 mm. Hg to 70 mm. in one minute, its effect being more toxic than that of 30 per cent. ether (Table II). To induce anaesthesia, at least 2 per cent. chloroform vapour is necessary, while 20 per cent. ether vapour is more than sufficient for that purpose.

It is thus obvious that ether possesses a great advantage over chloroform as regards its effect on heart muscle.

The work of Nicloux (16, 17) furnishes information as to the amount of ether and chloroform actually present in the blood at different stages of anaesthesia. He gives the following figures for the amounts present in 100 grams of blood:—

	Ether	Chloroform
At onset of anaesthesia	0·105 to 0·110 gram. ...	0·03 to 0·04 gram.
During deep anaesthesia	0·130 to 0·140 „ ...	0·04 to 0·05 „
After death from anaesthesia ...	0·166 to 0·175 „ ...	0·06 to 0·07 „

According to Dieballa (9), a percentage of ether less than 0·25 in the circulating fluid has no effect on the isolated dog's heart, while Derouaux (10), whose figures are lower than those of other observers, only occasionally observed slight weakening with 0·15 per cent. Dieballa never obtained stoppage with less than 2 per cent. On the other hand, Sherrington and Miss Sowton (18) frequently obtained stoppage of the isolated heart with less than 0·03 per cent. chloroform in the circulating fluid.

Thus the quantities of ether present in the blood during anaesthesia cannot produce paralysis of heart muscle; primary heart failure from this source is thus impossible in a healthy subject.

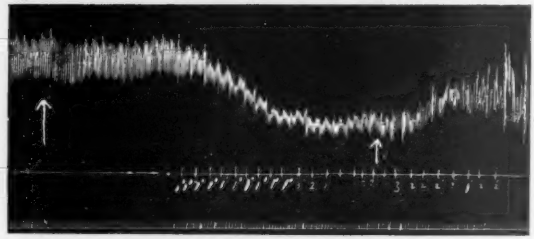

FIG. 3.—Effect of 10 per cent. Ether vapour on 'isolated' heart circulation. Blood pressure in this circulation taken in carotid. Drum stopped 27 times for periods aggregating 45 minutes.

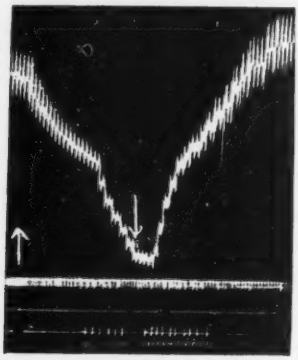

FIG. 4.—Effect of 19·5 per cent. Ether vapour on 'isolated' heart circulation. Blood pressure as in FIG. 3. Drum stopped 17 times for 10, 10, 10, 10, 15, 15, 10, 5, 5, 5, 5, 10, 10, 15, 15, 15, 30 seconds respectively.

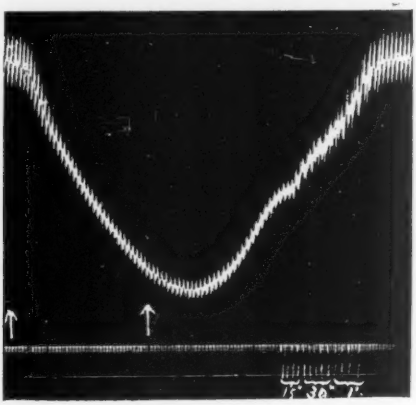

FIG. 5.—Effect of 30 per cent. Ether vapour on 'isolated' heart circulation. Drum stopped 4 times for 15 seconds, 4 times for 30 seconds, 4 times for 1 minute.

The Effect of Ether upon the Vagus Mechanism

There are, as far as I can find, no observations bearing on this point. When the important part the vagus plays in connection with chloroform anaesthesia is considered, the necessity for investigation of the action of ether on this mechanism becomes apparent. When air containing less than 30 per cent. ether vapour is administered to a dog, little or no slowing of the heart occurs. When more than 30 per cent. ether vapour is given, slowing is of frequent occurrence, and in rare cases the heart may actually stop. This has occurred only twice during the present series of experiments. The results on one occasion are described in the following experiment:—

Experiment. Dog, 7 kilogrammes. Morphia 0·3 grammes. By artificial respiration 30 per cent. ether vapour administered. At commencement of administration heart's rate was 136 per minute. In two ~~hours~~ min. ten ~~minutes~~ seconds the blood-pressure fell to 66 mm. and the heart's rate to 96. Two seconds after this the heart suddenly stopped. The ether was at once turned off, and in thirty-three ~~minutes~~ seconds the heart started again. The rate, however, remained slow for about five minutes.

Slowing of the heart does not occur if the vagi have been previously divided. If they are divided during a period of slowing, the rate at once increases. Ether rapidly depresses the vagus mechanism, so that inhibition of the heart by excitation of the vagus centre soon ceases to be possible. In a later stage of the experiment described above the continued administration of a large percentage of vapour did not cause inhibition a second time. Section of the vagi three and a half minutes after the first inhibition was not followed by any increase in pulse rate or blood-pressure. For the occurrence of cardiac inhibition with ether, a low blood-pressure seems essential. This doubtless accounts for the absence of vagus slowing when ether is administered by the closed method.

Early in ether anaesthesia, it is easy to arrest the heart temporarily by faradic stimulation of the vagus, but to permanently arrest the heart by such means seems impossible at any stage of the administration. In its effect on the vagus mechanism ether thus differs markedly from chloroform. When chloroform of 3 per cent. strength is administered to the dog vagus inhibition is of general occurrence; furthermore, under chloroform vagus inhibition may recur two or three times during the same administration ere the mechanism is too depressed to respond.

ERRATA.—On page 86, line 13, for 'two hours ten minutes,' read, 'two minutes ten seconds,' and line 16, for 'thirty-three minutes,' read, 'thirty-three seconds.'

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The Effect of Ether upon the Vascular System

In the intact animal simultaneous blood-pressure and plethysmograph records always indicated a marked vascular dilatation during the administration of ether.[1] Fig. 6 shows the result of giving 20·5 per cent. ether vapour in artifically respired air. In less than five minutes the blood pressure fell from 120 to 96 mm. Hg, while the tambour lever of the plethysmograph rose very considerably.

Experiments in vertical rotation of animals under deepening ether narcosis into the 'head-up' position also indicated marked vascular relaxation.

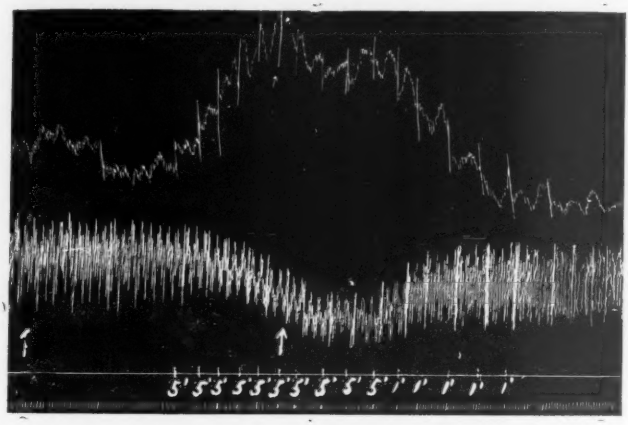

Fig. 6.—Fall of blood pressure, increase in bowel volume, on administering 20·5 per cent. Ether vapour to intact animal. Drum stopped for periods marked.

For instance, in an experiment where the blood-pressure had fallen from 98 to 88 mm. Hg, rotation into the 'head-up' position caused a further fall to 60 mm. Hg; when continued administration had reduced the pressure to 56 mm, rotation brought it to zero.

Since this vascular relaxation might be due to depression of the vaso-motor centre, of the peripheral vaso-motor apparatus, or of both, the effect of ether upon each of these was investigated. Although several observers have noticed vascular relaxation during administration of ether, they have not attempted to analyse the phenomenon.

1. In many experiments the tambour lever rose beyond the limits of the recording paper so that a complete record of its movements could not be obtained.

The Effect of Ether on the Vaso-motor Centre

By means of an artificial circulation defibrinated blood containing ether was delivered through the two internal carotids to the brain, which thus possessed a distinct blood supply from the rest of the animal. The blood-pressure in the systemic circulation and the changes in bowel volume were recorded.

The results showed that in the pre-anaesthetic stage, ether produced a marked pressor effect. When complete anaesthesia was attained this effect disappeared. In the fully anaesthetised animal, even the injection of pure ether into the carotid arteries was without effect on blood-pressure or bowel volume.

When the sciatic nerve was subjected to intermittent faradic stimulation under deepening ether anaesthesia, the pressor response was at first active, but as the anaesthesia deepened it became more and more sluggish and ineffective. In chloroform or ethyl chloride anaesthesia the pressor response persists for a longer period than under ether. With these agents, however (11, 12), depressor responses frequently occur when full anaesthesia is attained; indeed, with ethyl chloride they are as frequent as the pressor responses (13). I have never observed depressor effects in any of my experiments with ether. When it is given with limited asphyxia their occurrence is extremely improbable.

The Effect of Ether upon the Peripheral Vaso-motor Apparatus

To investigate this question, the double artificial circulation described by Embley and Martin (14) was employed. By means of one artificial circulation defibrinated blood was delivered to the isolated lungs of a dog, which were at the same time supplied by artificial respiration with air charged with ether vapour. The ether-charged blood received from the pulmonary veins was distributed by means of a second artificial circulation to a piece of bowel enclosed in an oncometer. The blood-pressure in this system was measured by the method described in the former paper.

The typical results observed are seen in the following experiment, in which 10 per cent. of ether vapour was administered:—

Experiment. Effect of ether on circulation through isolated loop of bowel.

An artificial circulation was carried on through lungs and loop of bowel of dog, a separate artificial apparatus being employed for each. As circulating fluid, the defibrinated blood of two dogs was used. By artificial respiration the lungs were ventilated with air containing 10 per cent. ether vapour.

A small loop of bowel was placed in an intestinal plethysmograph. Blood pressure in bowel circulation and bowel volume were recorded.

The blood-pressure before the administration, a constant flow having been attained, was 140 mm. Hg. Ten minutes after the ether had been admitted to the respired air the blood-pressure had fallen to 128 mm.; the tambour lever had risen 37 mm. The ether was then discontinued. In the next ten minutes the blood-pressure fell to 112 and the tambour lever had risen another 42 mm. Three minutes later the blood-pressure was 108 mm. and the tambour lever had risen above the recording paper. In another experiment (Fig. 7) ether vapour of 30 per cent. strength was administered for one minute forty-five seconds. The blood-pressure at the start was 196 mm. Hg. In two minutes it fell to 132 mm. The ether was now cut off, and in seven minutes the blood-pressure had regained its original level.

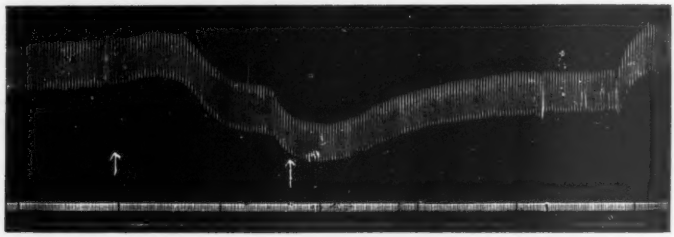

FIG. 7.—Effect of 30 per cent. Ether vapour on isolated bowel circulation. Blood pressure fell in 2 minutes from 196 to 132 mm., recovery to original level in 7 minutes.

In similar experiments, changes in the calibre of arterioles were demonstrated by measurement of the rate of blood flow from the veins before and during ether administration, the pumping force being kept constant. All the experiments performed showed that ether causes marked relaxation of the arterioles independent of the central vaso-motor mechanism.

Derouaux (10) obtained an increased rate of flow when he added ether to fluid circulating through the excised kidneys of a dog. From experi-

ments made with an artificial circulation across a capillary tube, he decided that this increase was due not to vaso-dilatation, but to a diminution in the co-efficient of friction of the liquid. He concluded that ether in no way modifies the calibre of the vessels apart from the action of the central nervous system. The results obtained by plethysmographic methods are in direct contradiction to this conclusion. When ether is introduced into the circulating fluid in the normal way, i.e., by means of the lungs, there is certainly marked dilatation from action on the peripheral vessels. In the preceding section I have pointed out what a small part the action on the central vaso-motor mechanism plays in the total effect.

The Effect on the Circulation of the Respiratory Changes Produced by Ether

Augmentation of respiratory movements is an obvious result of ether administration, both in clinical and experimental observations. The effect of this upon the circulation may be of importance. Augmentation of respiration if not excessive aids the circulation and increases the blood-pressure; if excessive it may have an opposite effect. Further, excessive ventilation washes out CO_2 from the blood and tissues, and this, as Yandell Henderson (15) has shown, causes a fall of blood pressure apart from mechanical effects. The effect of limitation of air supply in maintaining blood-pressure during ether anaesthesia is probably largely dependent on the fact that under such conditions the alveolar CO_2 tension is kept high, so that the tension in the blood remains at or above normal.

When respiratory paralysis from an over-dose of ether occurs—a result which I have several times observed in my experiments—the heart suffers from lack of oxygen and rapidly fails. Such an effect is seen in Fig. 1. In this experiment the *coup de grâce* to a circulation already weak from an over-dose of ether was given by deprivation of oxygen from cessation of respiration.

Conclusions

1. Ether administered with a plentiful supply of air in quantities sufficient to induce anaesthesia causes a fall of blood-pressure. This fall is readily counteracted by asphyxial stimulation such as occurs when the air supply is limited.

2. Ether in large concentration paralyses heart muscle. The paralytic effect increases with the strength of vapour administered, but not in a

direct ratio, since the effect increases more rapidly than the percentage of ether. While the percentage of chloroform present in the blood during anaesthesia is sufficient to paralyse the isolated heart, the percentage of ether has to be increased threefold before it produces any obvious adverse effect on the isolated heart. The percentage of ether present in the blood of dogs killed by an overdose of ether is not sufficient to produce any effect on the isolated heart. Primary paralysis of heart muscle from overdose of ether does not occur in ether anaesthesia; when heart failure occurs it is secondary to failure of respiration.

3. The vagus mechanism is at first stimulated by ether, and if an early and rapid fall of blood-pressure should occur during the administration of 30 per cent. or more of ether vapour, the heart may be inhibited; a sound heart, however, soon escapes from inhibition, permanent arrest in such case never occurring. The vagus mechanism is soon depressed by ether, so that in the latter stages of anaesthesia, inhibition cannot occur. Vagus inhibition does not occur unless the blood-pressure has fallen, and is therefore not seen when the closed method of administration is employed. In its action on this mechanism ether stands in strong contrast to chloroform. With chloroform, inhibition is frequent and persistent; with ether, infrequent and transitory.

4. Ether causes great relaxation of the arterioles. This effect is due almost entirely to its action on the peripheral vascular mechanism, since the vaso-motor centre is only very slightly depressed. It is to this relaxation of arterioles that the fall of blood-pressure during ordinary ether administration is mainly due. Mild asphyxiation, such as occurs in administration by the closed method, raises the blood-pressure, obviating the fall which would otherwise result. Chloroform and ether both cause relaxation of arterioles, but that due to ether is much more pronounced, though readily enough prevented. On the other hand depressor reflexes from stimulation of sensory nerves, which are not infrequent under chloroform, are never seen under ether anaesthesia.

5. Ether is in every way much safer than chloroform. A mild asphyxiation obviates the one disadvantage, the relaxation of arterioles, so that ether administered clinically by the closed method is a very safe anaesthetic.

I have again to acknowledge, with much gratitude, my indebtedness to Professor Osborne for his generous rendering of every assistance and for the use of the laboratory during this investigation.

Protocols

Fig. 1.—Effect of large percentage of ether on intact animal. Fall of blood-pressure, respiratory failure, heart failure. *Dog*, 9 kilogrammes. Morphia 35 grammes. Ether in large concentration administered throughout by open method. Respiration failed in 9 minutes 35 seconds. Circulation ceased in 10 minutes 10 seconds. Drum stopped eight times for 1 minute, once for 30 seconds.

Fig. 2.—Intact animal. Ether administered with limitation of air supply. Rise in blood-pressure and maintenance above normal for some time. *Dog*, 8 kilogrammes. Morphia 4 grammes. Blood-pressure and respiratory records. Ether administered by Clover's inhaler attached to tracheal cannula. Rise of blood-pressure from 110 to 124, 146, 150, and 146 by successive application (at 'on') and withdrawals (at 'off') of ether. Blood-pressure after these remained steady at 116 mm. Hg for 2 minutes. Ether then given by open method. Blood-pressure fell only 10 mm. in 6 minutes 5 seconds.

Fig. 3.—Effect of 10 per cent. ether vapour on 'isolated' heart. *Dog*, 8·3 kilogrammes. Morphia 0·4 gramme. $CHCl_3$ anaesthesia for preparation. All arterial trunks from aorta ligated except carotids, into one of which manometer was inserted, while the other carried the circulation. Pulmonary circulation intact. Artificial respiration. Blood-pressure 184 mm. Hg at start, fell in 27 minutes 5 seconds to 136 mm. Drum stopped several times for 1, 2, or 3 minutes. Blood-pressure recovered in 20 minutes to 174 mm. Hg.

Fig. 4.—Effect of 19·5 per cent. ether vapour on 'isolated' heart. *Dog*, 10 kilogrammes. Morphia 0·5 gramme. Carotid and lung circulation as in 3. Blood-pressure at start 186 mm. Hg. In 7 minutes fell to 74 mm., recovered to 210 mm. Hg in 3 minutes 30 seconds. Drum stopped many times during administration.

Fig. 5.—Effect of 30·2 per cent. ether vapour on an 'isolated' heart. *Dog*, 10 kilogrammes. Morphia 0·5 gramme. Carotid and pulmonary circulation as in 3. Blood-pressure fell from 200 mm. Hg to 68 mm. in 1 minute 20 seconds. Recovery of blood-pressure to 200 mm. took place in 9 minutes 30 seconds. Drum stopped several times for 10, 30, and 60 seconds.

Fig. 6.—Increase of bowel volume under ether. *Dog*, 8 kilogrammes. Morphia 0·4 gramme. Artificial respiration. Blood-pressure and bowel volume records. Ether 20·5 per cent. from 'on' to 'off' for 5 minutes. Blood-pressure fell from 121 to 96 mm. Hg lever recording bowel volume steadily rose during that time. In 9 minutes 15 seconds the blood-pressure and bowel volume had returned to their former levels. Drum was stopped several times for 5 and 1 minute periods.

Fig. 7.—Effect of ether on isolated bowel. Preparation as in experiment described on page 88. Ether vapour, 30 per cent., administered for 1 minute 45 seconds from 'on' to 'off.' In 2 minutes 10 seconds the pressure fell from 196 to 132 mm. Hg. Blood-pressure recovered to 196 mm. in 7 minutes after cessation of administration.

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ON THE CHEMISTRY AND BIO-CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF A SAPO-GLUCOSIDE OBTAINED FROM THE SEEDS OF *BASSIA LONGIFOLIA* (MOWRAH SEEDS).[1]

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Introduction

The seeds of *Bassia longifolia* are imported into Liverpool from India because of a valuable oil which they contain in large quantity. The oil is a clear yellow semi-solid fat or butter at ordinary temperature and is excellent for the manufacture of the better classes of soaps. It is expressed by pressure of the crushed seed while hot, and the residue, known as Mowrah meal, is the material which we have used for our work, as it contains all of the sapo-glucoside[2] in which we are interested on account of certain physiological actions closely resembling those of the cardiac tonics of the digitalis group of drugs. The meal, moreover, being almost fat-free, and already crushed, saves a good deal of preliminary labour which must be gone through if the whole untreated seeds are used as the experimental material.

The tree from which the seed is obtained is found in Central India, its habitat extending roughly in a belt about 120 miles broad from Bombay to Calcutta.

It is stated by Watts, in his 'Dictionary of Economic Products of India,' that the tree is of great importance to the natives and that any condition interfering with the Mowrah crop may cause a famine in the district. The flower is of a cream colour and appears in March and April,

1. The expenses of this research have been in part defrayed by a grant from the Government Grant Committee of the Royal Society.

2. We have introduced the word sapo-glucoside to designate the large number of substances belonging to the glucoside class which also show the properties of 'saponin,' and act physically like soaps. It is confusing that the name of one substance, saponin, should be applied to dozens of different compounds of different constitution. The term sapo-glucoside conveniently shows that these bodies belong to the class of the glucosides, and possess soap-like properties. To these soap-like properties attach other important powers such as haemolytics and cardiac drugs, which they share with soaps and bile salts.

before the leaves. The orange-coloured fruit, which has somewhat the appearance of an apricot and is from one to two inches long, ripens about three months after the flowers have fallen. Each fruit contains from one to four of the Mowrah seeds. These seeds yield the meal with which we have worked; they resemble in colour, size, and appearance small almond seeds.

Our attention was drawn to this product for two reasons, both connected with the presence of the sapo-glucoside. One reason was of commercial; the other of medical interest.

The usual commercial use made of the spent seeds used for obtaining oils for different purposes is to convert them into a basis for feeding-cakes for cattle—a purpose which they serve most admirably, as they contain a good deal of nutrient matter in an easily digestible form, and the process of crushing only enhances this nutritive value.

In the case of Mowrah seeds, however, the high market value of the oil is somewhat discounted by the difficulty of using the meal in appreciable amount for cattle-cake manufacture. For the sapo-glucoside in which we are mainly interested possesses such a bitter taste that cattle will not eat cake containing any high percentage of the meal. The extreme bitterness of the sapo-glucoside is shown by the fact that this is so, although the meal only contains approximately one per cent. of the sapo-glucoside.

The other irritant and poisonous properties of the sapo-glucoside which we are about to record have also made manufacturers chary of using the meal in cake manufacture on account of possible litigation.

As we shall see later, however, the sapo-glucoside when taken by the mouth is much less poisonous than when it is administered subcutaneously or by intravenous injection. Cattle or laboratory animals compelled to eat it in considerable amount do not show any obvious signs of poisoning, and in small amounts in cake it might possibly be more advantageous than deleterious.

The other reason which brought the Mowrah meal before us, was the effect of the fine dust from it, after it had been crushed and the fat extracted, upon the mucous membranes or any abrasions of the skin of workmen employed in manipulating it, especially in any way which involved stirring it up, such as shovelling.

On cut surfaces, or mucous surfaces, it acts usually as a most powerful irritant, preventing cuts from healing by producing inflammation, sloughing and erosion; and on the mucous surfaces it causes sneezing and

coughing in those not inured to it, with a hot irritating sensation in the throat, followed by catarrh and inflammation.

Our investigations soon demonstrated that both the bitterness of the meal and its irritant action were due to the same substance present in small amount in it. This body was then found to be a glucoside, and shown by its frothing in aqueous solution and powerful haemolytic action on red blood corpuscles to be a sapo-glucoside. Both the glucoside itself and its organic acid obtained on hydrolysis fortunately proved to have such solubilities in different solvents, as enabled us readily to separate them in a state of purity, and follow up both chemical constitution and physiological properties of the pure sapo-glucoside.

The interesting discovery was also made that the action on the cardiac mechanism of the sapo-glucoside very closely resembles that of the digitalis group of cardiac tonics, the heart being slowed and the strength of its beat increased by small doses.

According to Waller,[1] activity in digitalis preparations is associated with the presence of the physical property of frothing in the solutions; and, also, the action of saponin on a nerve-muscle preparation is similar to that of digitalis.

In our opinion, this large group of allied substances, hitherto called 'saponins,' which we have termed sapo-glucosides, owe their cardiac action to a physico-chemical action on the cardiac lecithides, similar or identical in kind to that which causes them all to produce laking of the red corpuscles by their soap-like action on the lecithide of the corpuscular stroma. The action is probably due to a molecular union, or adsorption, preceding a solvent action.

As far as we are aware, the particular sapo-glucoside with which we have here experimented has not been previously described or investigated, and we have ventured to name it Mowrin from its origin in Mowrah seeds, while the organic acid resulting from its hydrolysis we have called Mowric acid.

The work can be best described in the following sections, and, since we have not all been engaged in the work of each section, the names of the authors responsible are given under each sectional heading:—

A. Organic and physical chemistry.
B. General physiological properties.
C. Haemolytic action.
D. Action on the isolated mammalian heart.

1. *Proc. Physiol. Soc.*, Dec., 1908; *Journ. of Physiol.*, Vol. XXXVIII.

SECTION A. ORGANIC AND PHYSICAL CHEMISTRY

By Benjamin Moore and T. A. Webster

Preliminary investigation. Samples of the meal were extracted with hot water and hot alcohol respectively. The hot alcoholic extract was evaporated to dryness and the residue dissolved in water. Portions of aqueous and alcoholic extracts injected into animals both killed rapidly, showing that the toxic substance was soluble in both water and alcohol.

The material from both extracts was found to reduce Fehling's solution, and showed other reactions of a reducing sugar. On hydrolysis, by boiling with hydrochloric acid, a white gelatinous precipitate came down, rapidly setting into a jelly in even moderately dilute solutions. The filtrate from this precipitate was found to contain a still larger amount of sugar, and from this it was suspected that the active body was a glucoside.

The clear filtered alcoholic extract from the meal was treated with ether (1 vol. of alcohol to 2 vols. ether), which gave rise to a precipitate, as a white voluminous mass. This precipitate was filtered off rapidly and dried *in vacuo* over sulphuric acid. The product thus obtained was a *pale* yellow amorphous powder, soluble in water in all proportions and sparingly soluble in absolute alcohol.

Tested physiologically this substance was found to be intensely active. It possessed an exceedingly bitter taste, frothed strongly in aqueous solution, and laked blood corpuscles almost instantaneously. Injected into frogs it caused slowing of the heart, with rapid death.

A solution in water of this powder was found to give all the reactions of a glucoside. On hydrolysis by acid, it gave a white gelatinous precipitate, turning the whole fluid into a thick solid jelly, just as did the original extracts of the meal; and the filtrate showed strong reducing properties and gave a typical glucosazone. In addition to the insoluble acid and the reducing sugar, a volatile substance with a distinct smell resembling that of fusel oil was formed; the smell of this substance is most typical and is always observed when the glucoside is hydrolysed.

The sapo-glucoside, as precipitated by ether and taken up in water, gives a beautiful colour reaction, which appears to occur as a class reaction with all the sapo-glucosides. This colour is a fine purple, exactly like that given by Pettenkofer's test for bile salts, and is obtained similarly by the addition, either to particles of the powder or aqueous solutions of the same, of strong sulphuric acid.

With aqueous solutions, the test is best carried out in the contact-ring form in a test-tube, a beautiful purple zone developing at the junctional layer between the strong sulphuric acid and the aqueous solution. The same result is got by adding a few particles of the solid powder to strong sulphuric acid, when the acid turns first red and then a deep purple throughout. On dilution, the colour is precipitated out, as a dirty greyish precipitate, and it is insoluble in all the usual solvents.

The similarity of this test to the Pettenkofer test is remarkable, and, as we shall see later, furfurol, which is necessary in the Pettenkofer test, forms a part of the sapo-glucoside molecule, or at least is set free in quantity on hydrolysis of the mowrin.

We may also pause here to point out how similar the above separation is to the precipitation of the bile salts in the Plattner method for obtaining the bile salts. Both this sapo-glucoside and the bile salts are soluble in alcohol and in water, and insoluble in ether.

The above colour test is probably due to some common molecular grouping in both bile salts and the sapo-glucoside. Both the cholalic acid of the bile, and the acid of this and other sapo-glucosides, are complicated organic acids, as we shall see, unsaturated and hydroxylated, in that they can additively take up halogen or acetyl groups; all of them reduce the surface tension of water, as shown by the frothing they produce in aqueous solutions; all rapidly haemolyse red blood corpuscles; and all slow the beat of the heart.

The precipitate produced in the Mowrin solutions, on hydrolysis by acids, was easily soluble in alcohol. It was shown to be an organic acid by dissolving readily in water on treatment with alkali and reprecipitation on adding excess of acid again. It was also shown to have a low but quite definite neutralization figure.

This preliminary investigation having pointed out the method of attack, the preparation and purification of the mowrin was carried out as follows:—

Method of isolation. Two to three kilograms of the meal were taken and placed in three large beakers, the contents of which were treated successively with several quantities of cold absolute alcohol. Filtration was at first effected through calico, and the filtrate from beaker No. 1 was used to extract the meal in No. 2, and so on, to No. 3. Fresh alcohol being always added first to No. 1, in successive quantities, until No. 1 was about exhausted, as shown by absence of ether precipitation in a test of the alcoholic filtrate, when the meal in No. 1 was discarded, and so on.

The alcoholic extracts were then combined, and evaporated down to about one-third of their original bulk. The clear extract on cooling was treated with double its volume of ether, and the precipitated glucoside was then filtered off, by means of a Buchner funnel, well washed with ether, and set at once to dry *in vacuo* over sulphuric acid. It was found that if the alcohol extract was too concentrated, the glucoside, instead of coming down as a white flocculent precipitate, was thrown out as a brownish gummy mass, which adhered to the sides of the vessel with great tenacity. This appears to be due to the great attraction of the glucoside for water, in which it is soluble in all proportions, and takes up greedily from the air. Accordingly, when there is not a sufficient excess of absolute alcohol to hold firmly the small amount of water present, this comes out with the precipitated sapo-glucoside. So long as there is excess of alcohol, the glucoside is precipitated as a white flocculent mass. In its precipitation in this latter form the sapo-glucoside appears to hold in molecular union, or in some physical fashion, a large amount of alcohol or ether, for although the precipitated mass is very bulky it dries up to a very small amount. In fact, the precipitate rapidly becomes semi-fluid almost before it can be got into the desiccator, and *in vacuo* begins to boil, leaving behind a yellowish white body. This is then purified by repeating the process of solution in alcohol and precipitation with ether, until finally an almost pure white substance is obtained.

This substance is entirely organic, leaving no ash on incinerating on platinum foil. Qualitative tests, and a Kjeldahl analysis giving negative results, demonstrated that it contained no nitrogen, and by exclusive qualitative testing, it was shown to consist of only carbon, hydrogen and oxygen. After drying to a constant weight *in vacuo* over sulphuric acid, the substance was combusted with the following results:—

Combustion	No. 1,	Carbon =	51·90 %	: Hydrogen =	6·85 %	; Oxygen =	41·25 %
,,	No. 2,	,,	50·29	,,	6·67	,,	43·04
,,	No. 3,	,,	50·34	,,	7·32	,,	42·34
Average	...		50·84		6·95		42·21

The nearest empirical formula yielded by these figures is $C_{51}H_{84}O_{32}$; but analyses from different samples vary somewhat, and results from freezing point determinations, from bromination and from acetylation, yield quite different values. These variations we believe to be due to changes in amount of polymerization of the mowric acid, which appears to show great facility for condensing. This discussion will be resumed at the end of the description.

Properties of Mowrin. The sapo-glucoside, mowrin, is an amorphous white powder with a pale yellow tinge, soluble in all proportions in water and fairly soluble in methylic and ethylic alcohols, pyridine, aniline, and glacial acetic acid. The very strong solutions in water have the gum-like appearance of colloidal solutions, and the substance never separates as a discrete solid phase in equilibrium with clear solution, no matter how much it is concentrated. The aqueous solutions even at extreme dilutions give permanent frothes with the greatest ease.

The acid is set free on hydrolysis as a kind of hydrogel, and even a fraction of 1 per cent. concentration gives a completely solid jelly in the acid solution. For complete hydrolysis, a certain concentration of acid is required, for hydrolysis was found to be incomplete even after twelve hours with 1 per cent. hydrochloric acid. Boiling with acetic acid even up to full concentration did not give the characteristic precipitate of the mowric acid, and it was at first erroneously thought that acetic acid was not strong enough to induce the hydrolysis. It was found later, however, that the mowric acid is soluble with readiness in acetic acid, and then it was seen that the acetic acid had hydrolysed the mowrin but had held the mowric acid in solution, for the precipitate appears on diluting the solution boiled with acetic acid with a large excess of water.

Products of hydrolysis. Two grammes of mowrin were dissolved in 100 c.c. of distilled water, and hydrolysed by boiling with 50 c.c. of concentrated hydrochloric acid. In about two minutes the whole mass set solid from the separation of gelatinous mowric acid. This was diluted with distilled water and filtered, and the precipitate was thoroughly washed and then dried, *in vacuo*, over sulphuric acid.

This precipitate when first formed was of a pale pinkish white colour, but the colour rapidly darkened, and it was found that even with most careful manipulation and avoiding exposure to air it was impossible to preserve it in a white form. In the process of drying it shrunk to a mere fraction of its original bulk, and a quantity which had filled in gelatinous form a 500 c.c. beaker, when dried weighed less than one gramme. This bulky gelatinous character made work with it exceedingly difficult, and washing and filtering most tedious.

It was readily soluble in alkalies, and on acidifying was again thrown out in the same gelatinous form.

Solubilities of mowric acid. It is insoluble in water, ether, and cold hydrochloric acid, slightly soluble in hot acid and easily soluble in alcohol and glacial acetic acid. We were unable to obtain the acid in a crystalline

form. It was prepared in as pure a condition as possible, by precipitating the crude acid as above described, redissolving in alkali, reprecipitating with hydrochloric acid and washing thoroughly and drying. This product was then combusted with the following result:—

Amount of substance taken	=	0·1413 gram.
Yield of carbon-dioxide	=	0·3122 „
„ water	=	0·1121 „

This leads to precentages of C = 58·57, H = 8·81, O = 32·62. These figures approximate closely to the empirical formula $C_5H_2O_2$ (= mol. wt. of 101); but as the saponification figure obtained by titration of the acid with deci-normal alkali in several different experiments lead on the supposition of a mono-basic acid to a molecular weight value of about 570 to 600, it is clear that this figure must be multiplied by at least six.[1]

The acid is unsaturated, as shown by its changes on standing in the air, and it also is readily brominated and acetylated.

It is best brominated in solution in glacial acetic acid, and the brominated mowric acid is precipitated as a pale brown amorphous mass on diluting with water, and partially in minute crystalline form by evaporating the glacial acetic solution.

Estimated by Carius method, the bromine in the brominated product only amounts to 12·3 per cent. bromine. For an acid containing two atoms of bromine, which is about the lowest possible, this percentage of bromine leads to a molecular weight of 1,300, and indicates, therefore, a molecular weight of 1,140 for the mowric acid.

A titration of the bromo-mowric acid with deci-normal alkali gave, however, the result that 0·5 gramme only required 2 c.c. of alkali, leading to a molecular weight as mono-basic acid of 2,500, and indicating, therefore, with the above bromine percentage, a tetra-bromo-mowric acid.

An acetyl derivative prepared by boiling with acetic anhydride and precipitating with water gave, on saponification with alcoholic potash, neutralization of 30 c.c. of N/10 alkali by 0·5 gramme, which works out to 0·142 gramme of acetic acid to 0·358 of mowric acid, or 20·2 per cent. of acetyl in the acetylated acid.

This figure would give roughly three acetyl groups to each single molecule (at 600 mol. wt.) of mowric acid, or if there is a condensation of four of these, as indicated by the above bromination figures, then the more complex molecule of mowric acid would be able to take up twelve

1. In one experiment 0·154 gramme required for saponification 2·7 c.c. of N/10 alkali, leading on the supposition of a mono-basic acid to a molecular weight of 571; another experiment in which 0·2402 gramme were dissolved and titrated in alcoholic solution required 3·9 c.c. N/10 alkali, giving a molecular weight of 616.

acetyl groups or four bromine atoms; so pointing to two double linkages and to twelve hydroxyl groups in the molecule.

When these very variable indications are taken in conjunction with the molecular weights given by the cryoscopic method as seen below, it is obvious that the molecular weight must be left an open question.

The most obvious explanation which would correlate them all is that the mowrin molecule has a relatively simple constitution, but that the mowric acid on being set free polymerized very rapidly, as its physical character as a jelly also indicates.

The mowric acid gives the violet coloration with strong sulphuric acid, described above for the sapo-glucoside, and, as will be pointed out in a later section, possesses similar toxic properties.

It is a very stable body, not being perceptibly broken down into simpler bodies even by six hours' boiling with 20 per cent. sulphuric acid.

The filtrate after the hydrolysis was next examined qualitatively and quantitatively for the detection and determination of the remaining constituents of the mowrin.

The reducing sugar was found to be dextrose. It gave a typical crystalline glucosazone, melting at the proper melting point, and was distinguished from fructose by not reducing ammonium molybdate in acetic acid solution.

The third product of the hydrolysis, viz., furfurol, was first recognised by the pink colour given by the vapour with aniline acetate paper. A further test was the obtaining of the phloroglucide on distilling with hydrochloric acid and treating the distillate with phloroglucinol, when the typical dark coloured phloroglucide was obtained.

As furfurol can readily be obtained, under such circumstances, as a decomposition product of certain sugars, such as the pentoses, it remained doubtful whether the furfurol obtained by the hydrolysis was so to be accounted for or whether it was a true and direct cleavage product of the mowrin itself. Thorough fermentation with yeast of some hydrolysed mowrin was found to leave no reducing sugar behind, as it would have done had pentoses been present, for these are not fermentable by yeast. Hence, if the furfurol be due to hydrolysis of pentoses from the mowrin molecule, the breakdown must occur in the preliminary stage of all, when the mowrin molecule is first broken by the acid. For this fermentation experiment shows that there is no pentose present immediately after hydrolysis. So we may conclude that the furfurol is not formed in the subsequent process of distillation with acid.

Quantitative Estimation of the Hydrolytic Products

1. *Estimation of mowric acid.* One gramme of mowrin was dissolved in 50 c.c. of distilled water and boiled for one hour with 50 c.c. of strong hydrochloric acid. The precipitated acid was filtered off, well washed with hot water, dried at 105° C. to a constant weight and weighed. Various analyses with different samples of mowrin gave results lying between 35 and 40 per cent. of mowric acid.

2. *Estimation of sugar.* The dextrose was estimated by hydrolysing a weighed quantity of mowrin with weaker hydrochloric acid, filtering off the mowric acid, boiling the neutralized filtrate with excess of Fehling's solution, filtering the cuprous oxide off on to a Gooch crucible, oxidizing and weighing, as cupric oxide. The results in various samples varied between 25 and 41 per cent. of dextrose in the mowrin.

3. *Estimation of furfurol.* The furfurol was estimated by the method of Kröber and Rimbach.[1] A weighed quantity of mowrin was distilled from 10 per cent. hydrochloric acid until the distillate amounted to 200 c.c., distilling at the rate of five or six drops a minute.

One gramme of phloroglucinol dissolved in 200 c.c. of 10 per cent. hydrochloric acid was then added to the distillate and left standing over night. The precipitated phloroglucide was then filtered on to a Gooch crucible, washed with 100 c.c. of 10 per cent. hydrochloric acid and dried at 100° C. for four hours. The amount of furfurol can then be calculated from tables experimentally worked out and published by the authors in the above paper.

The analysis was only carried out in one experiment, and gave 14·4 per cent. of furfurol, in that sample of mowrin.

The complete figures for this sample were as follows:—

Moisture	=	3·1 % (dried at 105° C.)
Furfurol	=	14·4
Dextrose	=	41·2
Mowric acid	=	40·1
Total	=	98·8

Physical Chemistry

It was naturally expected from the foregoing results that mowrin would possess a very high molecular weight, and show no appreciable depression of the freezing point. For the molecular weight of the mowric acid lies at the lowest computation from its neutralization figure at about 600, and the brominated acid leads to a molecular weight of about four

1. *Zeitsch. f. angewandte Chemie*, Vol. XV, p. 477, 1902.

times this, that is, 2400, and the mowric acid forms only about 40 per cent. of the mowrin, so that, on the assumption of one molecule of mowric acid in one molecule of mowrin, the molecular weight comes approximately to about 6000.

We were, therefore, considerably surprised to find that the Δ of aqueous solutions leads to a molecular weight of only about 310 to 385 for the mowrin. Such a figure on the basis of 40 per cent. of mowric acid in the molecule would lead to a molecular weight of about 120 for the mowric acid.

An attempt to determine the molecular weight in glacial acetic acid solution demonstrated that so far from depressing the freezing point, the addition of the glucoside actually raised the freezing point, just as would an acid of higher melting point.

Experiment I.—A 5 per cent. solution of mowrin in distilled water was prepared, and the freezing points of this solution, and of the distilled water used in its preparation, were determined with a Beckmann apparatus as follows :—

Distilled water F.P.		Mowrin 5 % solution F.P.
2·659	...	2·340
———	...	2·332
2·650	...	2·328
2·650	...	2·334
2·650	...	2·342
———	...	2·336
2·650		———
		14·012 ÷ 6 = 2·335

$\Delta = 2{\cdot}650 - 2{\cdot}335 = 0{\cdot}315$

$\therefore$ Mol. Wt. $= 19 \times 5 \div 0{\cdot}315 = 302$

Experiment II.—Molecular weight determination for a 5 per cent. solution of mowrin in distilled water.

Distilled water F.P.		Mowrin in 5 % solution, F.P.
3·518	...	3·280
3·516	...	3·276
3·521	...	3·276
———		3·282
10·555		———
———		13·114
Average 3·518		———
		Average 3·278

$\Delta = 3{\cdot}518 - 3{\cdot}278 = 0{\cdot}240°$ C.

$\therefore$ Mol. Wt. $= 19 \times 5 \div 0{\cdot}240 = 395$

The lower molecular weight for the undecomposed sapo-glucoside is also suggested by the fact that it dialyses quite rapidly through parchment paper, which it would not probably do if it possessed the molecular weight of 6000 indicated for it by the bromo-acid.

The aqueous solution of the mowrin possesses also a fair amount of

electrical conductivity, which cannot be attributed to inorganic matter, as the substance leaves no ash, and contains nothing but carbon, hydrogen and oxygen. The reaction is neutral, so that there is no free organic acid or base present. This peculiar conductivity, therefore, is an enigma to us at present, just as is the apparently low molecular weight in aqueous solution.

The resistance of a 5 per cent. solution of mowrin is about four times that of a N/10 solution of potassium chloride. The freezing point determinations giving about 350 as an average, this 5 per cent solution would be about an N/7 solution; and it is remarkable that an N/7 solution of a glucoside should possess anything like one quarter of the conductivity of a decinormal solution of potassium chloride.

SECTION B. GENERAL PHYSIOLOGICAL PROPERTIES

By F. W. Baker Young and Benjamin Moore

The only previous reference we have been able to trace to physiological or toxic properties is contained in Watt's work already quoted. Under the heading of Medicinal Properties he states that 'the cake left after the expression of the oil is employed as an emetic and as a detergent. It is used to poison fish, and the smoke produced in burning the cake is reported to kill insects and rats.'

In 1908, one of us was called upon to attend numerous cases of cellulitis of the hands and feet of a particularly virulent type, and found in every case that the patients themselves considered their condition to be due to the fact that they were working with the meal known as Mowrah meal.

In these cases, the characteristics observed were acute inflammation at the site of entry of the Mowrah meal, supervening in a few hours from any injury received. There was intense pain, and although suppuration was slight, there was considerable destruction of tissue. In several cases the inflammation attacked the periosteum, causing necrosis in one or more phalanges.

Men working with the meal complain of headache, occasional vomiting, watering of the eyes, and haemorrhage from the nose. There seems to be a good deal of personal idiosyncrasy as to the effects of the drug. An aqueous solution of the meal approximately of one in five strength was made, and 1 c.c. of this was injected subcutaneously in a rat.

Within five minutes there was evident paralysis of the hind limbs. The respirations, which at first had been rapid, became deep, slow, and heaving, and there was evident 'air hunger.' Death occurred in about a quarter of an hour from respiratory failure. On examination after death, the peritoneal cavity was found to contain blood-stained serum; the intestinal area was injected with blood, and blood was observed in the lumen. No haemorrhages were found in the stomach, but the whole small intestine was covered over with haemorrhagic areas, and there was great destruction and sloughing of the mucous membrane and epithelium. An examination of brain and cord showed a congested condition of the meningeal linings.

Death followed from a similar subcutaneous dose in frogs and in rabbits, and in the latter similar intestinal lesions were observed.

In marked contrast with the above are the effects of feeding by the mouth. Considerable amounts, much larger than the above, and as great as the animals would eat, were given mixed with other food, such as in bran to rabbits, mixed with rice to rats, and enclosed in meat to dogs, without any noticeable injurious effects. The animals appeared to remain quite well. The pure glucoside in big doses was also given enclosed in meat to dogs without any observable deleterious effects.

The lethal dose for the rat in subcutaneous injection was determined by the following experiments, as well as the symptoms and effects of the drug. The pure sapo-glucoside was used for the injections. Subcutaneous injection of 1 c.c. of 2 per cent. solution of mowrin in normal saline (= 0·020 gram) into a rat weighing 119 grams, caused death in one hour twenty-four minutes. The first symptom observable was paralysis of the hind limbs; the breathing then became slow and laboured, followed by inspiratory gasping and finally stoppage of respiration; the heart still beat for some little time after cessation of respiration, and it was found at the post-mortem, engorged on the right side, as usual in an asphyxial death.

A careful examination was made about half an hour after death. A transparent fluid was found in considerable quantity in both the abdominal and thoracic cavities, deeply stained with free haemoglobin. The intestines were dark coloured and injected. The right heart, as above stated, was engorged with black coloured blood, and on opening contained a dark-coloured clot. The whole of the inner surface of the small intestine was closely covered over by haemorrhages.

A subcutaneous injection of half the above dose (i.e., 0·5 c.c. of 2 per

cent. solution = 0·01 gram) was given to a rat weighing 129 grams. Paralysis of the hind limbs first set in, followed by that of the fore limbs, and at the end of about forty minutes the animal lay upon one side, showing strong dyspnoeic respirations. The dyspnoeic condition gradually increased in intensity, and the animal died of respiratory failure at the end of two hours thirty-three minutes. An autopsy showed a similar condition to that described above in the case of the first animal.

The dose was halved again, and tested also subcutaneously in a rat of 150 grams, dosage now being 0·005 gram given in 1 per cent. solution. Death occurred with the same symptoms and appearances in three hours five minutes.

In a fourth rat, the dose was again halved, now amounting to 0·0025 gram to a body-weight of 124 grams. This dose caused paralysis and dyspnoea, but the rat recovered from the first effects, and next day was apparently all right. It died, however, next day, but did not show the above appearances of mowrah poisoning. No marked appearances of inflammation were noticeable in the intestinal area, and no haemorrhages. The lungs were collapsed, only just floated in water, and showed red hepatization. They were filled with a red sanguinous fluid. The animal appears to us to have succumbed to a pneumonic infection, perhaps introduced by the lowering action of the drug.

This dose of 0·0025 gram in a rat of 124 grams hence appears to be about the minimal lethal dose on subcutaneous injection of mowrin in this animal.

The pure glucoside administered by the mouth in much larger quantities along with food produced, as already stated, no noticeable effects. It was also given enclosed in meat, up to as much as 0·5 gram in a dog of 7·5 kilos., without producing any obvious effect. No sugar appeared in the urine, nor could any mowrin be detected in it by chemical testing. The heart beat and respiration remained unaltered as far as we could observe.

The peripheral effect upon the blood vessels, as shown by the perfusion of the blood vessels in saline in the pithed frog, is shown in the accompanying tracing (Fig. 1), kindly taken for us by Messrs. Foster, Jones & Wright. The effect is a marked constriction, and this result was confirmed in several concordant experiments. The lowering effect upon arterial blood pressure on intravenous injection in the intact anaesthetized animal about to be described cannot accordingly be set down either to direct peripheral action or, as seen on the blood pressure tracing itself, to decreased heart activity.

On intravenous injection in an anaesthetized dog, the drug causes a distinct fall in blood pressure (see Fig. 2), with such a dose as we gave there was no apparent action on the heart, although in the isolated and perfused heart the striking results recorded in Section D were obtained. The explanation of this apparent anomaly appears to us to be that in the intact animal the mowrin is seized and bound, and so rendered inert by serum proteins, and the lecithides of corpuscles and tissues, so that little or none is left over to attack the heart lecithides and produce the characteristic results. On the isolated heart perfused with saline there are no other substances save the heart lecithides and heart proteins present to meet the attack of the mowrin. It accordingly enters into union, probably of a molecular or adsorption type, with these bodies altering their bio-chemical balance, and so interfering with the heart-beat. In any case, on direct introduction into the circulating blood there is none of that profound action on the heart which might perhaps have been expected *a priori*, from the known action on the isolated and perfused heart.

Such a difference in action on the isolated heart and on the heart *in situ*, renders the former a more exquisite test for physiological action of a cardiac drug; but it should also be remembered, in relationship to dosage, that a drug is going to produce many-fold less action in the heart *in situ* than an equal amount produces upon a heart excised and perfused with saline. A similar action occurring entirely *in vitro*, is seen in the difference in laking power of haemolytic agents (such, for example, as these very sapo-glucosides) (*a*) upon corpuscles *in serum* and (*b*) upon corpuscles *in saline*. In the latter case one-hundredth part or less of the haemolytic will produce as great an effect as in the former.

We have here accordingly to distinguish between a good and delicate engine for research, and the natural production of physiological, pharmacological, or pathological effects.

We believe that in this difference in equilibrium as between saline and blood, in the case of such organic colloidal or semi-colloidal bodies which can unite specifically with proteins or lecithides, lies the cause of apparently conflicting results as between excision perfusion methods and testing with the heart *in situ*.

In the case of mowrin, for example, if the exceedingly minute trace in saline which alters the heart-beat be taken as a basis, and the dosage calculated either upon volume of blood or body-weight which should produce a similar effect in the body, a very much sub-minimal dose will be obtained. Also, we know from our injection experiments with mowrin

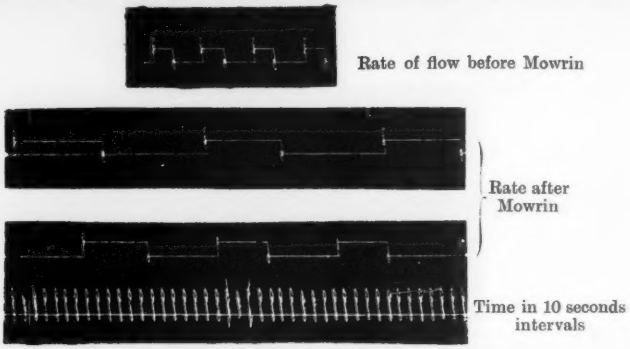

FIG. 1.—Rate of flow in peripheral blood vessels before and after Mowrin shown by outflow through the Schäferrttilter. The oscillations indicate outflows of 8 c.c. for each double oscillation shown electrically by upset of an oscillating trough (the Schäferrt-tilter). The concentration of the solution perfused was 1 in 100,000 of Mowrin. The animal used was a pithed frog perfused through aorta, outflow from *sinus venosus*.

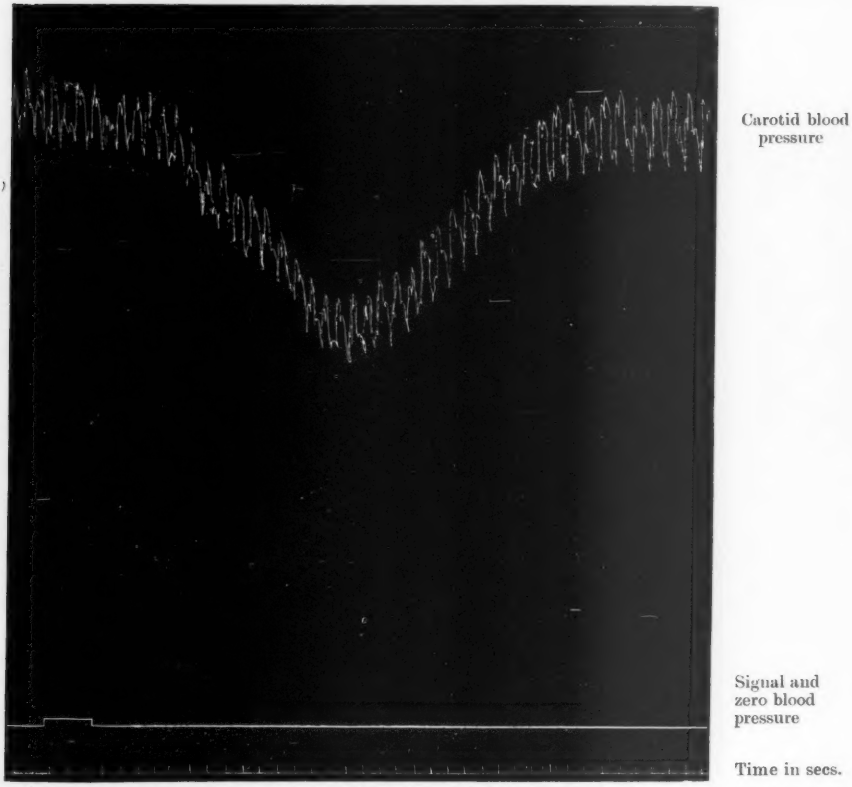

FIG. 2.—EFFECTS OF INTRAVENOUS INJECTION UPON ARTERIAL BLOOD PRESSURE.

Effects on arterial blood pressure of injection of 0·5 c.c. of 5 per cent. Mowrin (= 0·025 grams.) in dog of 5 kilograms. Anaesthetized by chloroform, cannulae in carotid artery and a crural vein—no other operative interference.

that death ensues from effects on the central nervous system; for death is preceded by paralysis and respiratory failure. A higher concentration is required to seriously affect the heart. Still, no better piece of physiological machinery than the isolated heart could be desired in order to demonstrate that the sapo-glucoside is toxic for cardiac muscle and to show the effects (see Figs. 4, 5, 7, 8 and 9, Section D) varying in quantitative amount with increasing dosage. As shown by Waller, skeletal muscle can similarly be employed as a pharmacological testing machine, and is somewhat simpler in its preparation. Here also the same remarks apply as in the case of the isolated heart, viz., that effective amounts, when dissolved in saline, will be obtained with much lower amounts than in natural perfusion in the blood; and hence that a marked effect upon the selected isolated tissue must not be taken to mean that that tissue will be the first to be attacked on exhibition of the drug in the intact animal.

SECTION C. HAEMOLYTIC ACTION

By Benjamin Moore

The action of mowrin, mowric acid, and bromo-mowric acid as haemolytic agents, has already been alluded to in a previous paper by Moore, Wilson and Hutchinson,[1] who found potent haemolytic effects with the sapo-glucoside, and also with its acid in considerably lessened degree, but scarcely any effect with the saturated bromo-compound.

Such haemolytic action is always found in all the sapo-glucosides in somewhat varying degree. It is similar in origin to that detergent action upon fatty or greasy stains, which has made them useful commercially as soap substitutes, and in the so-called dry cleaning of silken fabrics. This action they share with ordinary soaps, where the activity is chiefly due to oleates, and with the bile where the bile salts are the active constituents.

As we have already said, it is closely correlated with the action upon the heart, and the common cause of all this type of action, in soaps, bile salts, and sapo-glucosides is an adsorbing or molecular combining power for phosphatides or lecithides.

When union occurs between sapo-glucoside and erythrocyte lecithin, the result is haemolysis; when union occurs between sapo-glucoside and cardiac-tissue lecithin, the result is cardiac slowing, accompanied by

1. This Journal, Vol. IV, p. 346, 1909.

augmentation of the force of each beat with minimal or therapeutic doses, or by complete cessation in systole with larger doses. Although it has been proven by Moore and Roaf[1] that the anaesthetics such as chloroform and, to a much less degree, ether have similar affinities for proteins, it is not improbable that part of the action upon the heart tissue of anaesthetics may be due to a similar molecular attraction between lecithides and anaesthetics.

I desire, however, most distinctly and emphatically to point out that this is quite different from the Overton and Meyer view that the lecithides act as membranes to the cell of varying penetrability, and passable only to substances soluble in lecithides (so-called lipöids).

I have elsewhere shown that the lipöid membrane hypothesis breaks down both theoretically and practically, and while admitting both the importance and universality of lecithides in all cells, claim and definitely state that this is due *not* to their forming a membrane, surrounding or enclosing or forming a stroma to the other cellular constituents, but to their forming, along with protein and other organic substances, an integral and internal part of the living structure or bioplasm.

The action of drugs, such as the anaesthetics, or the sapo-glucosides with which we are here more intimately concerned, is hence a direct one upon the bioplasmic molecule, and not one due to interference with a membrane or the permeabilities of such membrane. In small doses, the haemolytic and cardiac drugs attach themselves to the bioplasmic aggregate (probably in part to the protein and in part to the lecithide) and by causing partial or total interlocking stop its physiological activity. In higher dosage, they decompose the bioplasmic aggregate altogether, disrupt it and carry off its lecithide. This leads to disruption of the entire cell such as we see in haemolysis.

Such a view has a firm basis in experimental facts, and correlates similar actions of drugs and substances which are at first sight quite dissimilar.

It correlates, for example, and explains upon a common basis, the laking of blood corpuscles by organic solvents such as ether or chloroform, with the similar action of the soaps, bile salts and sapo-glucosides; and places this set of phenomena in line with the similar action of all these bodies upon the heart. All this group, in varying quantitative degree, slow the heart-beat, lower arterial blood pressure, and lake blood corpuscles.

1. *Proc. Roy. Soc.*, B., Vol. LXXVII., p. 86.

Mowrin, as shown by the subjoined protocols, is an exceedingly powerful haemolytic. As will be observed, it shows no trace of that peculiar exaggerated action of lower concentrations discovered in this laboratory by McLean and Hutchinson[1] for certain bile salts and their derivatives, and called by them the *haemolytic paradox*. Instead of showing this the mowrin solutions show increasing haemolytic power, with increasing concentration, from doses which show no action up to doses which completely lake in ten seconds and are present in 7·68 per cent. concentration. The effect is continuous, and there is nowhere any break in the curve.[2]

This does not, however, mean a fundamental difference between bile salts and sapo-glucosides, for we are at present in this laboratory engaged upon a study of the whole group of sapo-glucosides; and we have found in *Hederin*, a sapo-glucoside of the leaves of Common Ivy (*Hedera helix*), an exquisite example of a haemolytic agent showing the paradox. The laking with very low concentrations being almost instantaneous and the *time* of laking increasing with *increasing* concentration of haemolyser.

The results of this investigation will shortly be published in a separate paper; they are mentioned here to indicate that the haemolytic paradox is shown by some haemolysers and not by others.

Haemolytic experiments. The experiments were carried out in the usual fashion in tubes containing a total volume of 5 c.c., made up of 1 c.c. of twice washed red blood corpuscles of the sheep made up to a 5 per cent. emulsion in 0·9 per cent. normal saline; *plus* a variable amount of a definite concentration of the mowrin dissolved in 0·9 per cent. normal saline; *plus*, again, a sufficient amount of 0·9 per cent. saline to make up to 5 c.c. total volume.

The experiments were carried out at ordinary laboratory temperature. The times count from final making up of the tubes, and were controlled by two observers. The first table shows the high degree of haemolytic power possessed by mowrin, and the absence of the paradox; the second table shows a balancing action against pig's serum which, as is well known, is haemolytic for sheep's red blood corpuscles.

The two haemolysers, instead of showing an additive action, retard each other. In other cases, two given haemolysers aid each other and show a purely additive effect, such, for example, as sodium oleate and sodium linoleate acting together.

1. *Bio-Chemical Journal*, Vol. IV, p. 369, 1909.

2. In a later experiment on the haemolytic powers of the sodium salt of the isolated mowric acid, the interesting fact came out that this body does not show any paradox when freshly made, the haemolytic power increasing continuously with the concentration; but if the solution is preserved stoppered up for about a fortnight, the paradox appears in a marked degree, and at the same time the solutions become more highly active. This change is probably due to chemical polymerization, and is being investigated further.

Mowrin behaves like oleates, linoleates, and bile salts in retarding pig's serum. It is therefore probable that mowrin would act reinforcingly in presence of these simpler haemolytics, and like them would balance against lecithides.

The results obtained are shown in the two Tables on pp. 114 and 115 and in Fig. 3, where the results of a different experiment are graphically plotted.

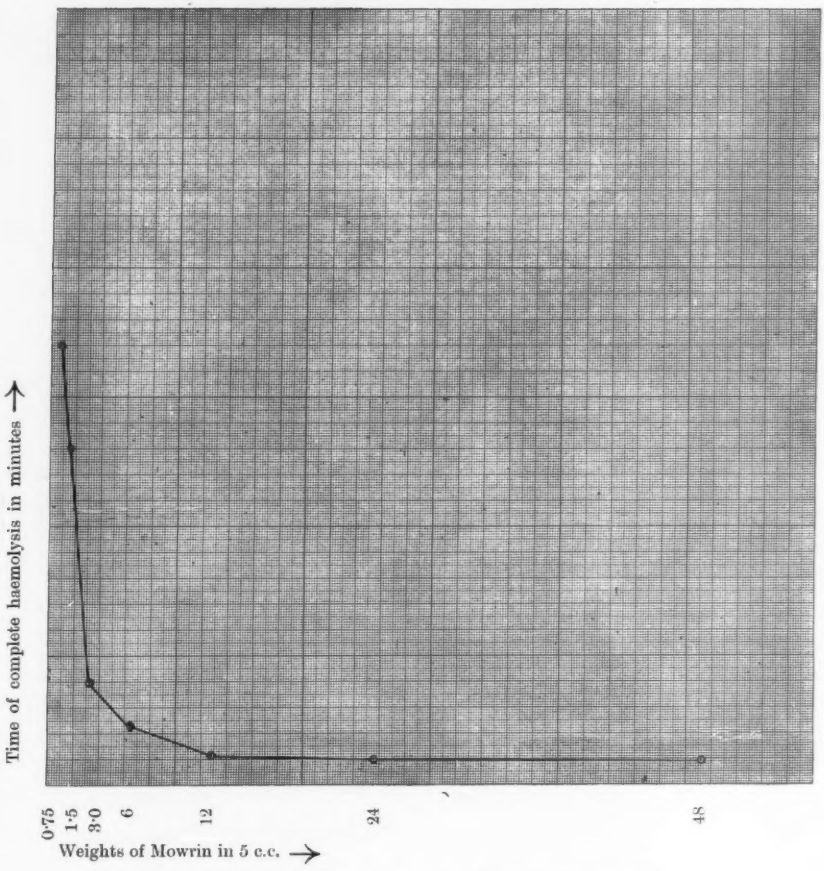

FIG. 3.—HAEMOLYTIC ACTION OF MOWRIN IN MILLIGRAMS.

I have to express my thanks to Messrs H. V. Foster, H. W. Jones and S. N. Wright, research students in this laboratory, who have rendered me much able assistance in carrying out the experiments of this Section.

TABLE I. HAEMOLYTIC POWER OF MOWRIN, AND ABSENCE OF HAEMOLYTIC PARADOX

Tube No.	Amount of mowrin solution in normal saline	Corresponding weight of mowrin in grams in the 5 c.c. of solution	Percentage of mowrin in tube when completely made up	Volume of normal saline added to make up to 5 c.c.	Result: Complete haemolysis in—
I ...	4 c.c. of 9·6%	0·384 gram.	7·68 %	Nil	10 sec.
II ...	2 „ „	0·192 „	3·84 „	2 c.c.	15 „
III ...	1·6 „ „	0·154 „	3·08 „	2·4 „	18 „
IV ...	1·2 „ „	0·115 „	2·30 „	2·8 „	20 „
V ...	0·9 „ „	0·086 „	1·72 „	3·1 „	23 „
VI ...	0·5 „ „	0·048 „	0·96 „	3·5 „	28 „
VII ...	4 c.c. of 0·6 %	0·024 gram.	0·48 %	Nil	30 sec.
VIII ...	2 „ „	0·012 „	0·24 „	2 c.c.	33 „
IX ...	1 „ „	0·006 „	0·12 „	3 „	50 „
X ...	0·5 „ „	0·003 „	0·06 „	3·5 „	1 min. 40 sec.
XI ...	0·25 „ „	0·0015 „	0·03 „	3·75 „	5 „ 47 „
XII ...	2 c.c. of 0·0375 % solution	0·00075 gram.	0·015 %	2 c.c.	8 min.
XIII ...	1 „ „	0·000375 „	0·0075 „	3 „	18½ „
	0·5 „ „	0·000187 „	0·0037 „	3·5 „	60 „
XIV to XVII ...	Contained still lower traces of mowrin, varying from 0·001 to 0·0001 %, and showed no haemolytic effect within four hours.				

One c.c. of a 5 % emulsion of washed sheep's red blood corpuscles in 0·9 % saline always added.

TABLE II. The balancing action of *mowrin* against *pig's serum* in the haemolysis of *sheep's red blood corpuscles*:—

Set I	5 % emulsion of sheep's red blood corpuscles	0·1 % solution of mowrin in normal saline	Pig's serum	Saline	Calculated weight of mowrin used	Times for complete haemolysis
No. 1	1 c.c.	2·0 c.c.	Nil	2·0 c.c.	0·002 gram.	10½ min.
„ 2	1 „	1·5 „	„	2·5 „	0·0015 „	15½ „
„ 3	1 „	1·0 „	„	3·0 „	0·001 „	23½ „
„ 4	1 „	**0·75** „	„	3·25 „	0·00075 „	**38½** „
„ 5	1 „	0·5 „	„	3·5 „	0·0005 „	1 hr. 37 min.
This series was placed in the incubator at 27° C. 3½ minutes after mowrin had been added to tube No. 1.						
Set II						
Serum alone	1 c.c.	Nil	1 c.c.	3 c.c.	—	20 min.
Set III		Placed in incubator immediately.				
No. 1	1 c.c.	2 c.c.	1 c.c.	1·0 c.c.	0·002 gram.	16 min.
„ 2	1 „	1·5 „	1 „	1·5 „	0·0015 „	19½ „
„ 3	1 „	1·0 „	1 „	2·0 „	0·001 „	29 „
„ 4	1 „	**0·75** „	1 „	2·25 „	0·00075 „	**45½** „
„ 5	1 „	0·5 „	1 „	2·5 „	0·0005 „	40 „
„ 6	1 „	0·25 „	1 „	2·75 „	0·00025 „	21 „
This series was placed in the incubator about 3 minutes after addition of haemolysers to the first tube.						
Control ...	1 c.c.	—	—	4 c.c.	—	Unchanged after 5 hours

The notable point is that at No. 4, Set III, 0·75 c.c. of 0·1 % mowrin, plus 1 c.c. of pig's serum, require 45½ minutes to produce complete laking, while an equal concentration of mowrin lakes in 38½ minutes, and an equal concentration of pig's serum lakes in 20 minutes.

SECTION D. ACTION UPON THE ISOLATED MAMMALIAN HEART

By S. C. M. Sowton

The method of perfusing an isolated heart through its coronary vessels is too well known to need description. The tests were made upon the hearts of rabbits, the animals used being from six to nine months old. The fluid used for perfusion was Locke's modification of Ringer's solution (without the sugar) supplied under constant oxygen pressure. Two flasks of the fluid were prepared, to one of which was added a given amount of the substance to be tested; the heart was allowed to settle down into a steady rhythm, and when this had continued for some time the drugged fluid was substituted for the normal Ringer's solution and the flow of it was continued throughout the experiment. The heart-beats were recorded in the usual way.

An orientating experiment (Fig. 4) with the glucoside at a strength of 1 in 2000 resulted in the arrest of the ventricle in four and a half minutes. The record of this very short experiment is not without interest, as it gives in a compressed form effects afterwards more conveniently observed with weaker solutions of mowrin and of mowric acid. The tracing shows the contractions increasing in size, then diminishing as the toxic action of the glucoside takes effect, this latter phase being accompanied by the final rise in tonus, which is so characteristic an end-effect with drugs belonging to the digitalis group, when used in a similar manner. Upon the coronary vessels the effect of mowrin at this strength was constriction so great as almost to arrest the flow of fluid.[1] A similar effect has occasionally been obtained with certain tinctures of digitalis when used at high percentages.

The next illustration (Fig. 5) is from the record of an experiment with mowrin much more diluted, namely, at 1 in 100,000. Slowing in the rate of beat with increase in its size is to be noticed, while rise of tonus becomes very marked as the experiment proceeds. At intervening strengths the glucoside gave similar results, but as these experiments form a less complete series than those made with mowric acid, attention is drawn more particularly to the latter experiments. They were ten in number, and were made from one 'brew' of the acid, whereas in the case

1. In Section B, p. 107 (see also fig. 1), it is shown that a similar constriction is produced in the peripheral blood vessels when dilute mowrin in Ringer's solution is perfused through the vessels.

of the glucoside two preparations were used, and they may have differed slightly.

For convenience of comparison, the record of a digitalis experiment is given in Fig. 6; it shows the well-known effects. The normal rate of beat, which was 152 per minute, is reduced throughout[1] and increase in amplitude of contraction is evident during the first half of the experiment. Later, tonus rises, and at the end of twenty-six minutes the ventricle is arrested in a state of tonic contraction. The tincture was used at a strength of 1 in 200. The highest strength at which mowric acid was used was 1 in 5000 (Fig. 7); as compared with the digitalis tracing, the similarity of effect is sufficiently striking; the normal beat is slowed during the first part of the experiment and the amplitude of contraction increased. Later, tonus rises gradualy, till, at the end of twenty-six minutes, the ventricle is arrested in tonic contraction.

The effect of mowric acid at 1 in 10,000 is shown by Fig. 8. The normal rate of beat of 136 per minute is gradually slowed; in twenty-five minutes it is 100 per minute; in fifty-three minutes it has fallen to 86 per minute; while still later the fall has reached 76 per minute, a reduction of 44 per cent.; this was the most marked reduction observed in any experiment. Increase in amplitude of beat is well shown, expansion as well as contraction being more complete, and tonus rises during the latter part of the experiment. In another experiment at this strength the reduction in rate of beat amounted to 29 per cent., and the size of contraction was increased by a third. Experiments were also made with the acid at 1 in 20,000 and 1 in 50,000; the slowing was less pronounced, but the increased size of contraction was always very well marked. In some cases this increase was preceded by a short-lasting diminution, such as is sometimes seen to follow the exhibition of the digitalis drugs.

In Fig. 9 the effect of the acid at 1 in 100,000 is shown; the slowing in rate and augmentation in size of beat are both evident. The experiment lasted one hour ten minutes and shows only the earlier phase of activity of the acid, that which in speaking of digitalis action would be called the 'therapeutic stage.'

At 1 in 200,000, the lowest strength used, the limit of activity of mowric acid appeared to be nearly reached; we have, however, but a single experiment at this strength. Some slight slowing was produced during the hour that the experiment lasted, at the end of which time the solution was exhausted.

1. With many tinctures of digitalis, and with all, so far as we know, of squill and strophanthus, the period of rising tonus is accompanied by a quickened beat, and the heart tends to pass into the condition of delirium.

Omitting the last experiment in which the slowing effect was very slight, the reduction in rate of beat, taking the average of nine experiments, amounted to 21 per cent. This is below the average diminution observed for tincture of digitalis, as given by Haynes,[1] who puts it at 30 per cent., the tincture being used at constant strength. In the present experiments, however, the strength of mowric acid varied, as has been seen, from 1 in 5,000 to 1 in 100,000, and a strict comparison cannot, therefore, be drawn.

The augmentation in size of contraction was in these experiments very marked, the average increase amounted to 64 per cent.; in three cases it was over 100 per cent. It was more marked with the acid than with the glucoside.

It has thus been shown that the action of mowric acid upon the isolated heart resembles that of the drugs of the digitalis group, alike in the earlier or 'therapeutic stage,' and in that later phase which is marked by a rising tonus. It is during this last stage of activity that irregularities of rhythm occur when the digitalis-like drugs are used, and the irregularities observable are of two types: that characterised by a dropping out of ventricular beats, and the more profound disorganisation of rhythm known as delirium cordis. The dropping of ventricular beats was observed when mowric acid was used at high strengths; it occurred in the two experiments at 1 in 5,000 and in one of those at 1 in 10,000. Delirium cordis did not occur in any experiment made with the acid, and one instance of it only was observed in the experiments with mowrin. In respect of their disorganising properties, therefore, the mowrah preparations would appear to resemble digitalis more nearly than they resemble squill or strophanthus. Strophanthus used at a strength sufficient to stop the beat in four and a half minutes, as is done by mowrin (Fig. 1), would, it may be said almost with certainty, have given the final quickening of beat which ends in delirium. Such an effect is much less certain with digitalis, many tinctures of which, as has been pointed out elsewhere,[2] can be used at high strengths without giving any trace of delirium.

In respect of strength, mowric acid is very much stronger than tincture of digitalis; how much stronger, the experiments so far made are not numerous enough to determine with any exactness. But judging by such indications as are available, one would say, roughly speaking, that the acid is some twenty-five times as strong as a good tincture. Taking

1. 'Pharmacological Action of Digitalis,' etc., this Journal, Vol. I, No. 2, p. 69.

2. 'Some Experiences in the Testing of Tincture of Digitalis,' *British Medical Journal*, February 6, 1908.

as a criterion of strength the time required to stop the ventricular beat, it is found that with mowric acid at 1 in 5,000 the mean of the time of arrest in two experiments was twenty-two minutes. In the experiment with digitalis at 1 in 200 (Fig. 3) the ventricle was stopped in twenty-six minutes, which is just above the average time of arrest for that strength of the drug as calculated from a large number of experiments.

In some of the experiments under consideration, though not in all, the heart became more oedematous than is usual in similar perfusions with the digitalis drugs; that at least is the impression derived from direct inspection of the heart.

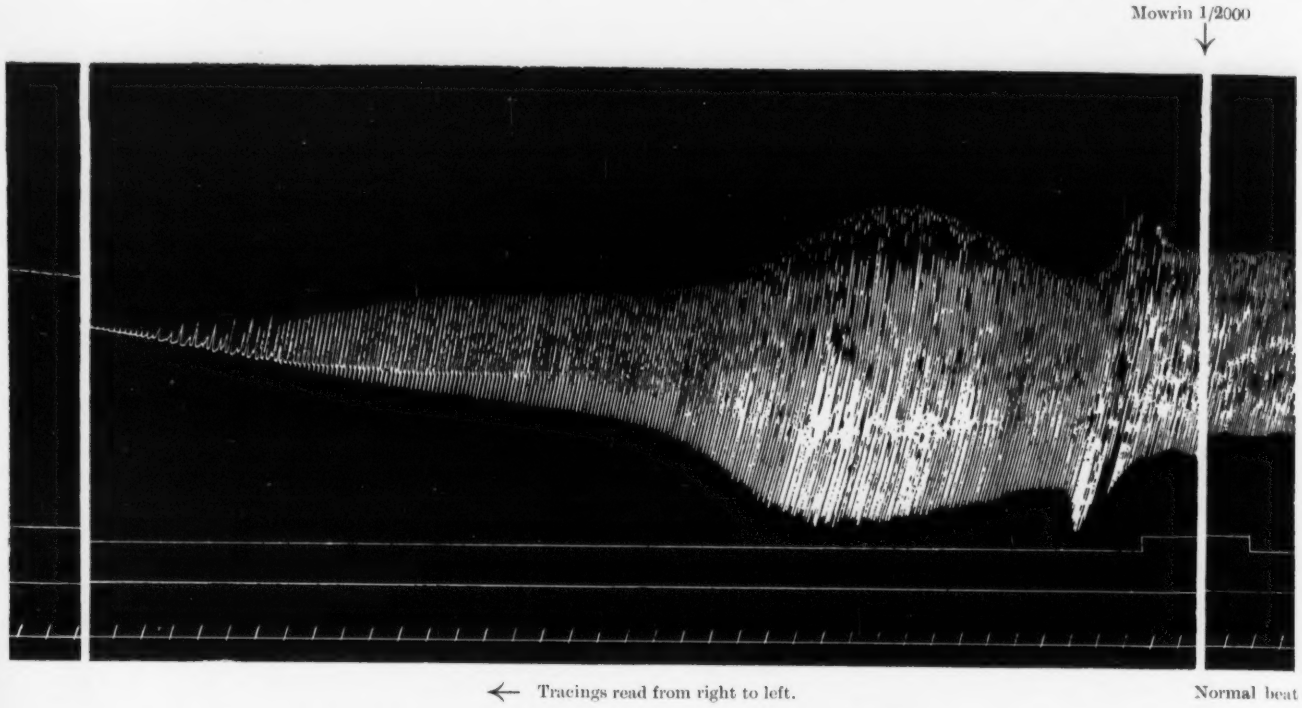

FIG. 4. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowrin at 1 in 2000. The ventricle is arrested in four and a half minutes, in tonic contraction.

In this and in the following figures the line immediately below the heart tracing is the electric record of the movements of the 'Schäfer tilter' by means of which the rate of flow can be measured. In this particular tracing the constricting effect is so great that the flow is almost arrested. The oscillations are well seen in the succeeding figures. The lowest line marks the time at intervals of 5 seconds.

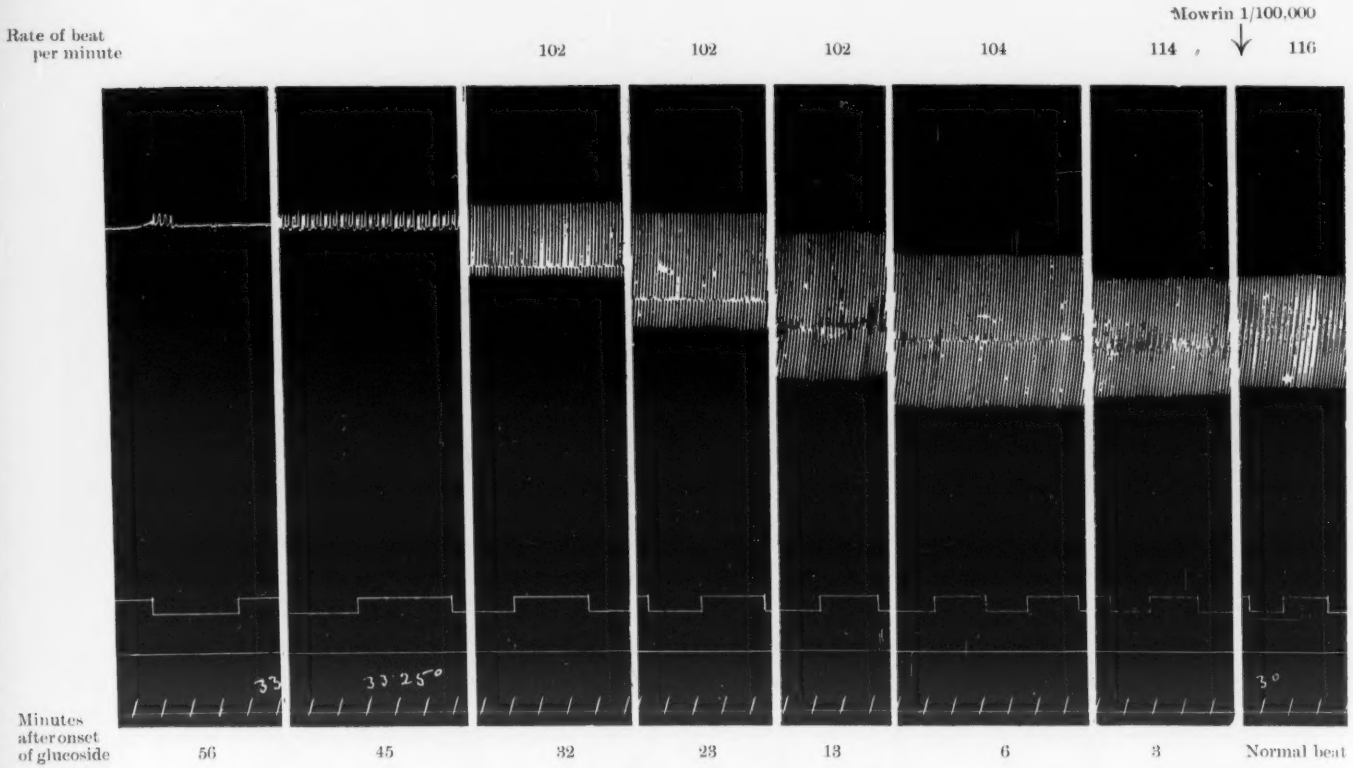

FIG. 5. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowrin at 1 in 100,000. The beat is reduced in rate and increased in size. The ventricle is stopped; a state of strong tonus.

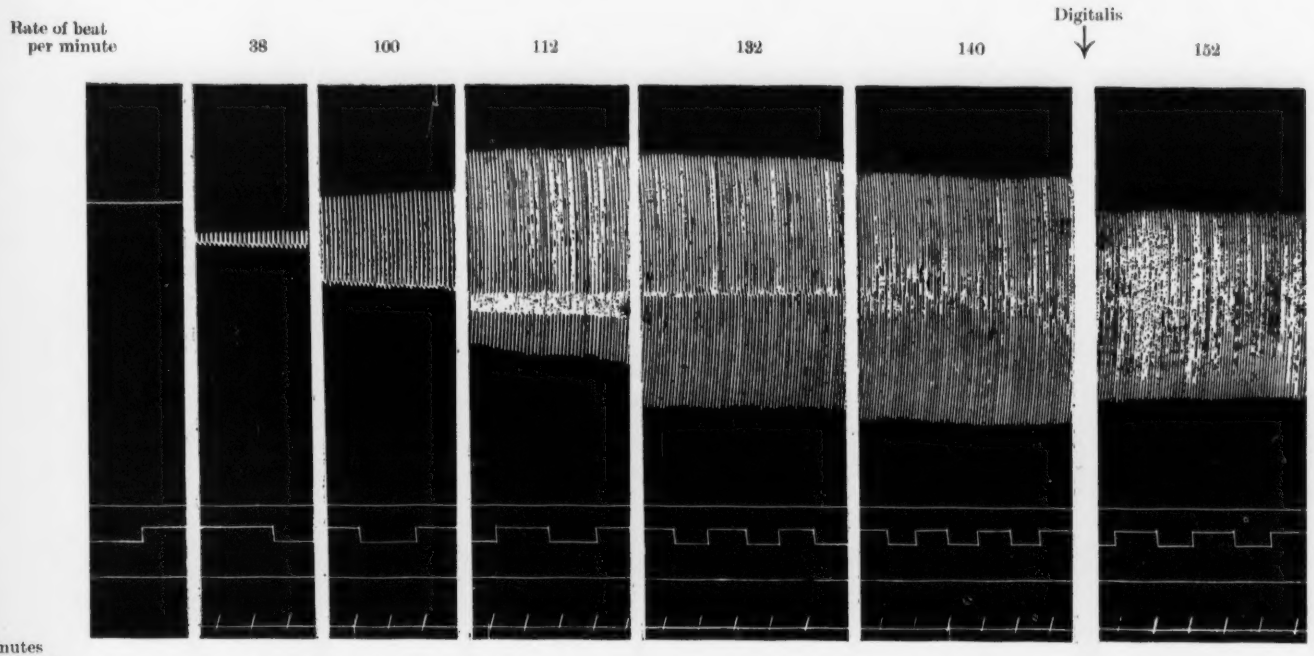

FIG. 6. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Tinct. Digitalis at 1 in 200. A piece of normal tracing is first shown; the succeeding portions of the record show the effect of the drug at intervals during the course of the experiment; in the last portion, twenty-three minutes after onset of the drug, the beat is only just visible and tonus is very high.

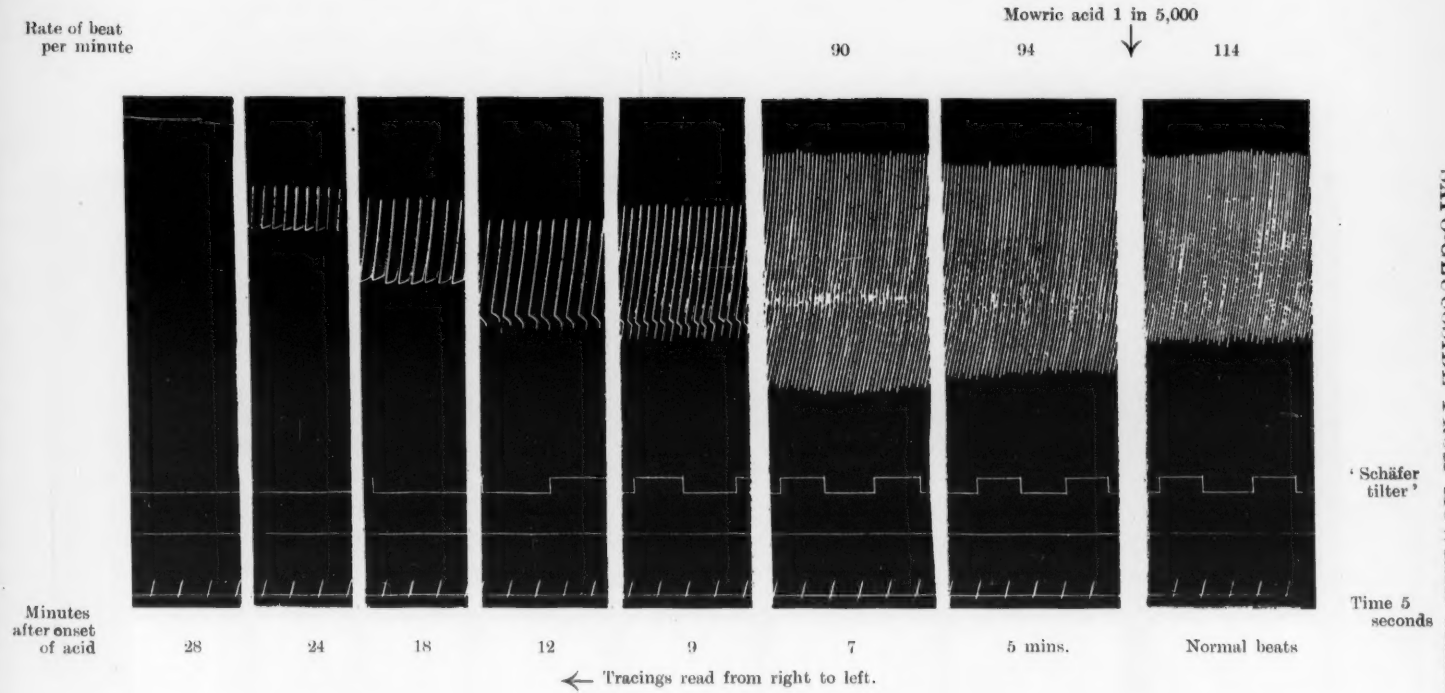

FIG. 7. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowric acid at 1 in 5,000. Slowing of beat is shown, and increase in force of contraction.

* At this part of the experiment the auricle was giving two beats to one beat of the ventricle; later there were several auricular beats to one ventricular beat. The ventricle is arrested in a state of strong tonus.

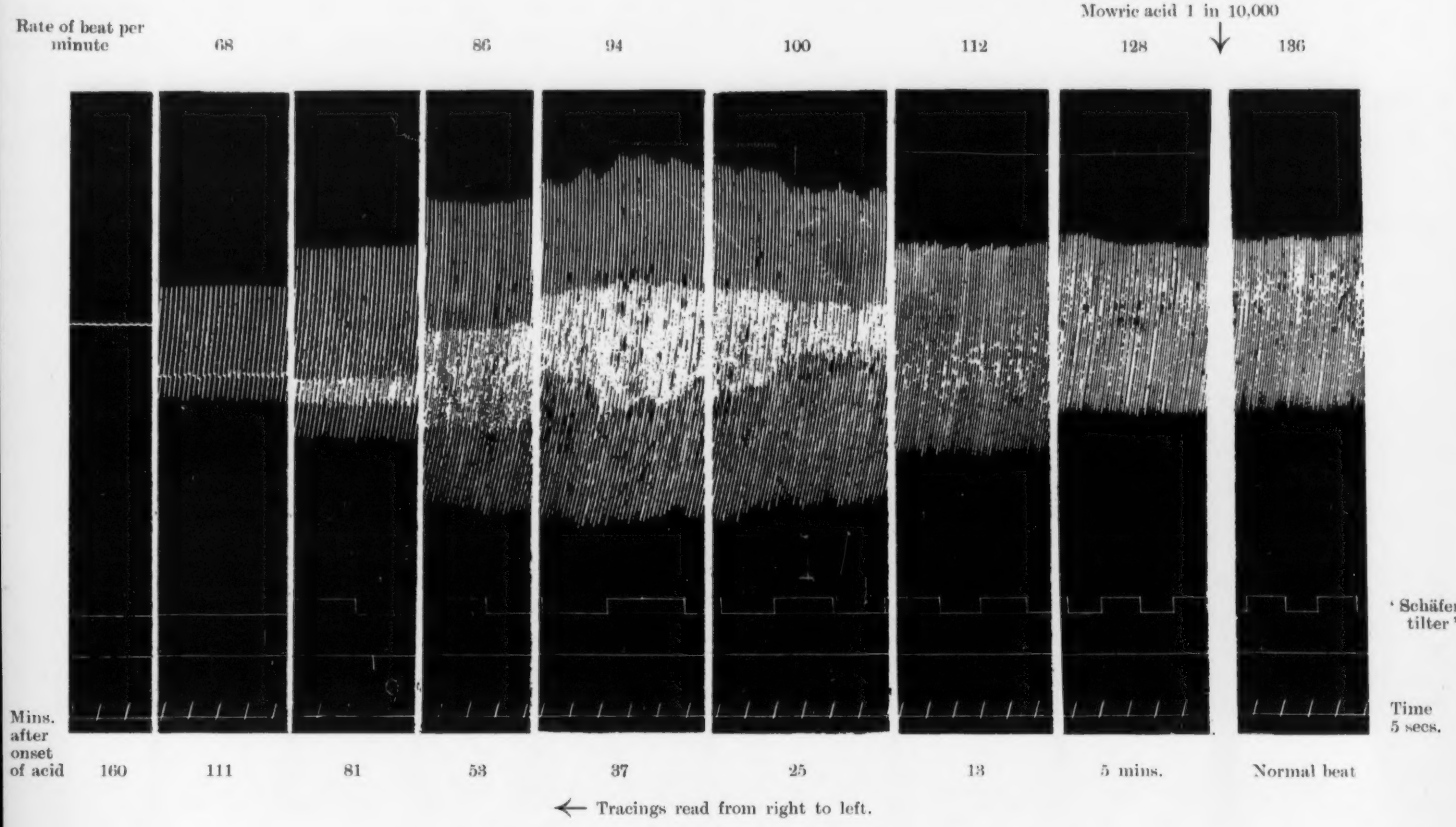

FIG 8. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowric acid at 1 in 10,000. Slowing in rate of beat and increase in force of contraction is shown. Tonus rises in the latter part of the experiment. The ventricle is arrested at the end of two hours forty minutes.

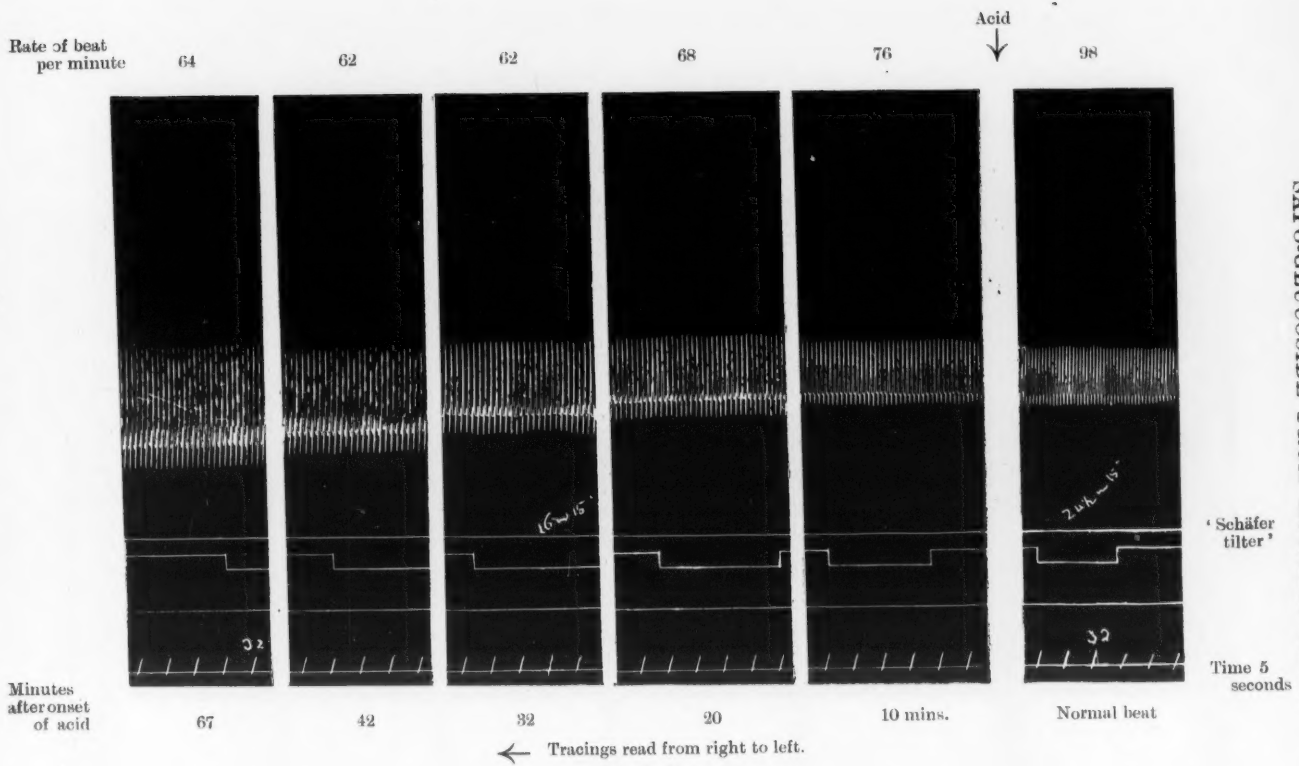

FIG. 9. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowric acid at 1 in 100,000. The beat is reduced in rate and increased in size. The experiment was stopped in one hour ten minutes, and shows only the earlier effects of the drug.

ON THE INFLUENCE OF THE PANCREAS ON THE GLYCOLYTIC POWER OF MUSCLE

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Since Minkowski and von Mering published twenty years ago the results of their investigations on pancreatic diabetes and showed that some internal secretion of the pancreas played an important part in the mechanism of the metabolism of sugar in the body, many investigators have tried to discover the method by which the internal secretion worked.

Certain observations by O. Cohnheim[1] in a series of papers in 1903-5 seemed to throw light on this question. He found that while neither fresh muscle juice nor pancreatic extract had alone any marked tendency to the destruction of sugar *in vitro*, a mixture of the two possessed such an action in a marked degree; and he further appeared to have demonstrated that the relationship of the two factors was similar to Ehrlich's conception of ferment and amboceptor; the muscle moiety being thermolabile, insoluble in alcohol and readily destroyed by any adverse conditions; the pancreatic moiety, on the other hand, thermostable, soluble in alcohol, and when in excess having an inhibitory power.

Arnheim and Rosenbaum,[2] Hirsch,[3] Dewitt,[4] and Hall[5] have published papers in confirmation of his observations, but these have been contested by Claus and Embden,[6] McGuigan[7] and others, and, unfortunately, the results obtained clinically by pancreatic feeding in diabetes have not been such as to confirm the hopes built on his observations.

As a preliminary to an intended investigation of this clinical failure I attempted to repeat Cohnheim's results, but found that in my hands no satisfactory results could be obtained.

Preparation of Extracts

At first slaughter-house muscle and pancreas obtained within half-an-hour to one hour after death were used, warm and not in rigor. The muscle was cut up into small pieces and well washed in cold tap-water, then pounded in a mortar with sand and expressed by a hand-press as completely as possible; usually water was added to the residue and it was then further expressed, and these washings added to the fluid obtained from the first expression. The pancreas was treated in the same way, but the expression was not carried far, as only a few cubic centimetres were required for each experiment. One hundred grammes of muscle was found to yield about 30 to 40 c.c. of juice by first expression; 5 to 7 c.c. of pancreatic extract is equal to 1 gramme of fresh pancreas.

The instruments and apparatus used both in extracting the juices and in the further course of the experiments were sterilised by boiling or by steam, except the pipettes and measuring flasks, which were thoroughly washed in water and then dried by alcohol. The juices were measured into flasks, roughly equivalent amounts of sterile solution of dextrose added to each; about 5 or 6 c.c. of 10 per cent. solution of phosphates (nine parts of di-sodium to one part of mono-sodium phosphate) was added to preserve neutrality, and 2 to 5 c.c. of toluol, the amounts in each experiment were then made up to constant volume with boiled cold water. The concentrations were, approximately, extract of 600 grammes muscle, extract of 20 grammes pancreas, 1·5 to 2 grammes dextrose, 10 to 20 grammes phosphate, 40 to 60 c.c. of toluol per litre.

Parts of the mixtures were diluted to the required proportion and estimated directly by Pavy's method; the rest incubated at 37° C. for twenty to twenty-four hours, and again estimated. The fluid in the incubated flasks was tested in most experiments as regards alkalinity, sweetness, presence of a surface layer of toluol, and sterility in aerobic organisms, at the end of the experiments. The first three conditions were present in all; a note of culture results is made in the experiment. The end-point of the estimation with Pavy was rather obscured by a marked biuret reaction in many experiments and rarely by blood pigment; fortunately as a rule the same errors came into parallel estimations and so cancel each other. After a time I came to look on the biuret reaction in slight degree as a rather favourable indicator of the end-point: there was a fairly definite point at which the last tinge of blue vanished and a pure pink colour only was left, and this point was determinable to 0·1 c.c. = 0·005 gramme error per 100 c.c. The point at which the biuret reaction

itself finally disappeared was very variable, sometimes a further 0·2 c.c., sometimes 5 c.c. of solution being required, and the amount further varied with the dilution, the rate of addition and the degree of ebullition to a marked and inconstant degree.

Series A.—Slaughter-house muscle and pancreas, obtained within one hour of death; expressed juices used; phosphate solution and toluol added with dextrose; sugar estimated before and after without removal of protein.

Experiment I.—Pig.

	MJ †	PJ †	Amount of solution	Sugar per litre Before	After	Loss of sugar Per litre	Per cent.	Per 40 c.c.	Cultures, colonies, and nature
a	25 c.c.	0 c.c.	in 40 c.c.	2·1604	1·6822	0·4782	21	0·019	—
b	25 c.c.	2 c.c.	in 40 c.c.	2·1874	1·0566	1·1308	51·5	0·045	—
c	0 c.c.	2 c.c.	in 40 c.c.	2·1552	1·8648	0·2904	13·5	0·016	—

Percentage excess of joint action (P+M) over sum of actions of its constituent actions + 17

† MJ = Muscle Juice. PJ = Pancreatic Juice.

Experiment II.—Pig.

	MJ	PJ	Amount of solution	Sugar per litre Before	After	Loss of sugar Per litre	Per cent.	Per 50 c.c.	Cultures, colonies, and nature
a	20 c.c.	0 c.c.	in 50 c.c.	2·3243	2·0270	0·2973	12·7	0·0148	0
b	20 c.c.	3·5 c.c.	in 50 c.c.	2·1583*	1·2362*	0·8321	38·6	0·0416	0
c	0 c.c.	3·5 c.c.	in 50 c.c.	2·3041	1·6529	0·6512	28·2	0·0325	1 mould

Percentage excess of joint action (P+M) over sum of actions of its constituent actions − 2·5

Experiment III.—Sheep.

	MJ	PJ	Amount of solution	Sugar per litre Before	After	Loss of sugar Per litre	Per cent.	Per 60 c.c.	Cultures, colonies, and nature
a	30 c.c.	0 c.c.	in 60 c.c.	1·695	1·61	0·085	5	0·005	0
b	30 c.c.	5 c.c.	in 60 c.c.	1·43*	1·575	−0·14	−10	−0·008	0
c	0 c.c.	5 c.c.	in 50 c.c.	1·94	1·88	0·06	3	0·004	0

Percentage excess of joint action (P+M) over sum of actions of its constituent actions − 18

Experiment IV.—Sheep.

	MJ	PJ	Amount of solution	Sugar per litre Before	After	Loss of sugar Per litre	Per cent.	Per 60 c.c.	Cultures, colonies, and nature
a	15 c.c.	0 c.c.	in 60 c.c.	1·92	1·44	0·480	25	0·027	0
b	15 c.c.	2 c.c.	in 60 c.c.	2·00	1·47	0·530	26	0·035	0
c	0 c.c.	5 c.c.	in 50 c.c.	2·24	2·03	0·110	5	0·007	1 culture

Percentage excess of joint action (P+M) over sum of actions of its constituent actions − 1
(P* = loss by 2 c.c. PJ).

The following experiment is included in this series for comparison; human muscle and pancreas; P.M. eighteen hours after death.

Experiment V.

	MJ	PJ	Amount of solution	Sugar per litre Before	Sugar per litre After	Loss of sugar Per litre	Loss of sugar Per cent.	Loss of sugar Per 50 c.c.	Cultures, colonies, and nature
a	22·5 c.c.	0 c.c.	in 55 c.c.	1·733*	1·684*	0·085	5	0·0042	—
b	22 5.c.c.	3·5 c.c.	in 55 c.c.	1·852*	1·129*	0·724	39	0·0362	—
c	0 c.c.	3·5 c.c.	in 50 c.c.	1·753	1·125	0·629	35	0·0314	—

Percentage excess of joint action (P+M) over sum of actions of its constituent actions – 1

* Indicates cases in which excessive haemoglobin or biuret reaction rendered estimation difficult.

Comments on Series A

In all this series the destruction of sugar by the action of pancreas and muscle together gave the highest actual and percentage value, with the exception of Experiment III; but on further analysis it is seen that the sum of the separate actions of muscle and pancreas alone is in only one instance (Experiment I) less than the amount of the action of pancreas and muscle together. This difference is shown below each experiment.

The pancreas alone in Experiments I, II and III showed a very high glycolytic figure contrary to the results obtained by previous observers, and for this reason, in spite of the negative results of the cultures used in Experiment B, an attempt was made to maintain a higher degree of asepsis in the work. In the next two experiments, C and D, a considerably lower figure was obtained for the pancreatic glycolysis, but also a lower one for the parallel observations in the presence of muscle.

In Experiment III an actual increase in sugar value, as measured by the reduction power, was obtained for the first time by me; this was at first attributed to some error in manipulation or possibly to an error in estimation due to the strong biuret reaction of the control; but as the same effect occurred in later experiments of mine and also in practically all the other recorded series, this was probably no error, and its reason will be discussed later.

This series, then, excepting Experiment I, gave no confirmation of Cohnheim's results, and so the use of alcoholic extracts of boiled pancreas was begun in the hope that by that a higher degree of glycolysis might be obtained, as stated by Hall and by Cohnheim, and also that a lessened chance of error from protein and bacterial contamination would exist, since on each occasion only the muscle would need expression, the sterile pancreatic extract being prepared beforehand.

The extract was made from the pancreas used in Experiments I, II, III and IV, Series A. The residue of pancreas and of expressed juice,

after a sufficient quantity for the experiments had been obtained, was again thoroughly pounded and ground with sand and water and evaporated nearly to dryness on a water bath; the residue was collected and extracted with successive portions of 95 per cent. alcohol; four to six lots of alcohol were used, each for one or more days; the successive portions of alcohol were mixed together, and, where necessary, reduced by heating on a water bath until of such a strength that 1 c.c. = 0·2 to 0·4 gramme pancreas.

In Series B, Experiments VI, VII and VIII, the extracts used were those of Series A, Experiment I and Experiment II, which are called respectively P.E. (i) and P.E. (ii). After that the four extracts made were mixed together and used for the remainder of the series (as P.E. (iii)). The muscle juice, toluol and sugar, and general course of the observations was as in the first series.

SERIES B.—Fresh muscle juice and alcoholic extract of pancreas from laboratory animals used: dextrose, toluol, and phosphate solution.

Experiment VI.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS.

				Sugar per litre		Difference in Sugar			Cultures	End reaction to litmus
	MJ	PE (i)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.	Per experiment	Aerobic	
a	30 c.c.	0 c.c.	in 60 c.c.	1·695	1·610	−0·085	− 5	−0·005	Sterile	Alkaline
b	30 c.c.	+2 c.c.	in 60 c.c.	1·538*	1·850	+0·312	+20·3	+0·019	Sterile	Alkaline
c	0 c.c.	3·5 c.c.	in 60 c.c.	1·560	1·370	−0·190	−12	−0·011	Sterile	Alkaline

Percentage excess of joint action over separate actions − 32.

Experiment VII.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS *a*.

				Sugar per litre		Difference in Sugar			Cultures	End reaction to litmus
	MJ	PE (i)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.	Per experiment	Aerobic	
a	15 c.c.	0 c.c.	in 60 c.c.	1·92	1·44	−0·48	−25	−0·027	Sterile	Neutral
b	15 c.c.	2 c.c.	in 60 c.c.	1·90	1·70	−0·20	−10·5	−0·012	Sterile	Alkaline
c	0 c.c.	2 c.c.	in 50 c.c.	1·515	1·44	−0·07	−4·6	−0·004	Sterile	Alkaline

Percentage excess of joint action over separate actions − 20.

Experiment VIII.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS *a*.

				Sugar per litre		Difference in Sugar			Cultures	End reaction to litmus
	MJ	PE (ii)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.	Per experiment	Aerobic	
a	15 c.c.	+0 c.c.	in 60 c.c.	1·92	1·44	−0·48	−25	−0·027	Sterile	Neutral
b	15 c.c.	2 c.c.	in 60 c.c.	1·92	1·49	−0·43	−22·4	−0·026	Sterile	Alkaline
c	0 c.c.	3 c.c.	in 50 c.c.	1·63	1·47	−0·16	− 9·8	−0·010	Sterile	Alkaline

Percentage excess of joint action over separate actions − 12·4

NOTE.—In each of above experiments separate estimations of each sub-division (*a*, *b* and *c*) were made before and after incubation, as in Series A. In the remainder of this series this procedure was not followed, as the pancreatic extracts had little reducing power, and so the addition caused little error. See estimations *a* and *b* in each of above.

Experiment IX.—Pig's Muscle. Juice expressed as in previous experiments; sugar solution, phosphate solution, and toluol added, and the whole diluted with sterile water and well shaken. Strength such that every 60 c.c. contained the juice of 10 grammes of muscle. Estimated reducing power of 60 c.c. was equal to 0·119 gramme sugar (the same mean value being given by the estimation of two separate portions diluted to four times the volume), that is 1·98 grammes per litre. Separate portions of 60 c.c. were measured into sterile flasks, and the desired amount of pancreatic extract added to each. This extract contained the extract of about 3 grammes of pancreas in 10 c.c., and had a glycolytic power of 0·002 to 0·003 gramme of sugar per cubic centimetre.

Experiment IX.—

	MJ	PE (iii)	Amount of sugar corrected for dilution by pancreas: Per experiment Before	Per experiment After 24 hrs.	Corrected to per litre original Before	Corrected to per litre original After 24 hrs.	Difference in sugar in grms.: Per Experiment	Per cent.	Per litre	Per kilo. muscle
a	Juice of 10 grammes of muscle in 60 c.c. of alkaline sugar solution	+0 c.c.	0·119	0·124	1·98	2·08	+0·005	+ 5	+0·10	+0·6 gm.
b		0·5 c.c.	0·119	0·112	1·98	1·87	−0·007	− 6	−0·11	−0·7 gm.
c		1 c.c.	0·119	0·117	1·98	1·95	−0·002	− 2	−0·03	−0·2 gm.
d		2 c.c.	0·119	0·107	1·98	1·78	−0·012	−10	−0·20	−1·2 gm.
e		3 c.c.	0·119	0·135	1·98	2·27	+0·016	+13	+0·29	+1·7 gm.
f		4 c.c.	0·119	0·143	1·98	2·37	+0·024	+19	+0·39	+2·4 gm.
g		6 c.c.	0·119	0·147	1·98	2·46	+0·028	+22	+0·48	+2·9 gm.
h		8 c.c.	0·119	0·136	1·98	2·26	+0·017	+14	+0·28	+1·7 gm.
k		10 c.c.	0·119	0·141	1·98	2·35	+0·022	+18	+0·37	+2·2 gm.

Reactions all alkaline after twenty-four hours, had excess of toluol on surface, and smelt quite sweet. No cultures taken.

Experiment X.—As Experiment IX, but incubated for forty-eight hours.

	MJ	PE (iii)	Amount of sugar corrected for dilution by pancreas: Per experiment Before	Per experiment After	Corrected to per litre original Before	Corrected to per litre original After	Difference in sugar in grms.: Per Experiment	Per cent.	Per litre	Per kilo. muscle
a	Juice of 10 grammes of muscle in 60 c.c. of alkaline sugar solution, containing toluol in excess	0 c.c.	0·119	0·130	1·98	2·17	+0·011	+10	+0·19	+1·14
b		0·5 c.c.	0·119	0·101	1·98	1·68	−0·018	+15	−0·30	−1·80
c		1 c.c.	0·119	0·139	1·98	2·31	+0·020	+16	+0·33	+1·98
d		2 c.c.	0·119	0·103	1·98	1·72	−0·016	−13	−0·26	−1·56
e		3 c.c.	0·119	0·127	1·98	2·12	+0·008	+ 7	+0·14	+0·84
f		4 c.c.	0·119	0·028	1·98	0·47	−0·091	−76	−1·41	−8·46
g		6 c.c.	0·119	0·076	1·98	1·27	−0,043	−36	−0·71	−4·26
h		8 c.c.	0·119	0·087	1·98	1·45	−0·032	−28	−0·53	−3·18
k		10 c.c.	0·119	0·022	1·98	0·37	−0·097	−81	−0·61	−3·66

Reactions all alkaline excepting *f*, which was markedly acid. All smelt quite sweet, had toluol on surface and no signs of organic scum (moulds, etc.). Aerobic cultures of *f* yielded no growth.

Comments on Series B

Experiments VI, VII, and VIII. Pancreatic extract has a glycolytic power of its own of 6 to 10 milligrammes dextrose per gramme of pancreas in twenty-four hours; fresh muscle juice a glycolytic power equivalent to from 8 to 20 milligrammes of dextrose per gramme of muscle in twenty-four hours; that is to say a fractionally larger power.

In mixtures of the two in the proportion of 0·6 gramme pancreas to 25 to 50 grammes of muscle, less glycolytic power than the sum of that of the constituents was generally apparent; whether from some antibody, or from the inhibitory action of excess of the glycolytic factor of the pancreas, or from veiling of the glycolysis by the formation of some breakdown product with power of reducing Pavy's solution.

Experiments IX and X. In these experiments it is seen that autolysis of muscle alone can lead to the formation of reducing substance; that the presence of pancreatic extract in large amount leads to increased formation of reducing substance, at any rate in the first twenty-four hours. Smaller amounts of pancreatic extract (0·15 to 0·6 gramme per 10 grammes muscle) led at first to a glycolytic action which reached as a maximum 1·2 grammes of sugar destroyed in twenty-four hours per kilogramme of muscle with 60 grammes pancreas, or corrected for the autolytic increase of reducing contents to 1·8 grammes sugar per kilogramme muscle in twenty-four hours.

Since the glycolysis was greater with 60 grammes pancreas per kilogramme muscle than it was with 15 grammes per kilogramme, it would appear that the small value of this factor was not due to any inhibitory action of excess of pancreatic extract. Also the total glycolysis is not nearly high enough to be comparable to the amount of sugar metabolised in the body during life.

On longer incubation (Experiment X), a great deal of variation is seen, the autolytic production of reducing bodies in the presence of large amounts of pancreatic extract is replaced by a large glycolytic action, while with smaller amounts the results become variable. In spite of the absence of any signs of bacterial or organism action, I cannot help feeling that some such action was possibly present.

This increase of reducing power first seen in Series A, Experiment III, and Series B, VI, and so very manifest in Series B, IX, X, led me to read the literature of the subject carefully once more, looking for this effect, and I found that the complication had occurred to previous observers.

Cohnheim did not obtain it in the series first communicated, in which he obtained his extract by freezing the muscle and then pulverising it, and in which he frequently mixed his pancreas and muscle from the start. He obtained it very frequently when he used normal saline solution, or other solutions, as oxalate and Ringer's solutions, for his extracts, or where he used such solutions to extract muscle, and also where he carried

on the extraction at temperatures above 0° C. It also appeared on the addition of pancreas in excess, blood serum and pancreas to slightly glycolytic muscle, or small amounts of pancreas to highly active muscle.

Claus and Embden, using saline solution to obtain constant concentration in their experiments, obtained a similar increase in several experiments.

Hall obtained an increase in two experiments only when using the juice of the fresh pancreas alone; never with muscle or muscle and pancreas, though working with large amounts of pancreas in many experiments.

Thinking that the presence of glycogen in the muscle might be a reason for the appearance of this complication, it was attempted at a later stage to estimate the amount of glycogen present in the muscle juice used by comparing its reducing power before and after boiling with hydrochloric acid. In none of the cases in which this was tried were any signs of glycogen breakdown observed, and it can therefore be assumed that the glycogen in the muscle changes rapidly and completely into sugar in the process of extraction, and so does not add to the sugar contents of the fluid during the later incubation. The experiments in which I had thus eliminated the glycogen factor showed, on incubation, only some very slight increase in glycolytic power (maximum 0·2 gramme per kilogramme muscle), and so are not very conclusive. I incline, however, to the belief that the increase is not due to the formation of dextrose from glycogen, but is an apparent one due to the formation of protein breakdown derivatives with reducing powers, on account of the fact that it generally appears in the presence of a large amount of pancreatic extract or juice, even though an alcoholic extract of boiled pancreas has not as a rule a high tryptic power.

In support of this compare Experiments IX and X, Series B, where the muscle alone yielded an apparent increase of 1·14 grammes dextrose per kilogramme after forty-eight hours, while in the presence of pancreatic extract there were increases equivalent to 2·9 and 2·4 grammes of dextrose per kilogramme muscle in twenty-four hours.

Other observers do not seem to have attempted to find any explanation for this factor, which greatly adds to the difficulties of the experiment, and which, when glycolysis is obtained, must be considered in comparing the observed power of glycolysis with the amount of glycolysis required for the metabolism of sugar in the body.

In answering Claus' and Embden's criticisms, Cohnheim alludes to the fact that in some of the experiments where he used saline, the

addition of pancreatic fluids seemed to reduce the inherent power of glycolysis of the muscle, and ascribes this to some inhibitory influence of the sodium chloride on the activating power of the pancreas, the nature of which he makes no attempt to explain.

It occurred to me that the work of Cole,[8] Schutz,[9] and others on ferments possibly held out an explanation of this fact. They showed that the nature of the ions in the solutions of ferments had a very considerable influence on their activity, and sodium was especially seen to have large inhibitory powers. Cole found that in some cases where sodium salts had an inhibitory effect ammonium salts had an accelerating effect, and this was tried in one case (compare Series C, Experiments XVII and XVIII, Nos. c and d), but without any result. I did not proceed further on those lines, since I was by then convinced that the production of reducing bodies, which undoubtedly occurs, might be favoured by such methods of extraction and so would quite account for the lessening of apparent glycolysis.

Very slight variations in the conditions of experiments on autolysis or protein digestion may lead to very divergent lines of breakdown, and while this factor cannot be eliminated, it must cause great difficulty in any work on the glycolytic influence of the pancreas by methods such as we have been using; and I feel that some such factor has to be reckoned with in such cases as that recorded by Cohnheim in his Experiment XX, Paper No. 2:—

a	Muscle alone gave glycolysis	=	0 sugar
b	Muscle + pancreas 8 c.c.	=	−107 mg. sugar
c	Muscle + pancreas 8 c.c. + serum 7 c.c.	=	− 75 mg. sugar
d	Muscle + Serum 7 c.c.	=	− 50 mg. sugar
e	Muscle + 4 c.c. pancreas + 3 c.c. serum	=	−100 mg. sugar

Cohnheim suggests that the serum contains the same activator as the pancreas for the glycolytic ferment of the muscle; that 8 c.c. pancreas contains the optimum amount of activator for the amount of muscle used (100 grammes); that 7 c.c. of serum contains sufficient activating body to partially inhibit the action; and, finally, that 3 c.c. serum and 4 c.c. pancreas again contain the optimum amount of activator.

Surely, if this be so, 3 c.c. of serum are equal to 4 c.c. of pancreas for the purposes of the experiment, and if that is right the activating effect obtained by 7 c.c. of serum should be almost of an optimum value; on the contrary, either it is below maximum, since it only causes half the maximal glycolysis, or else it is far over the optimum (6 c.c. should, strictly speaking, be optimum and equivalent to 8 c.c. pancreas), and has

a marked inhibitory effect. But this latter cannot be true, since 7 c.c. serum in excess reduces the maximum glycolysis by only 25 per cent., so 1 c.c. would scarcely reduce it by 50 per cent.

Moreover, Cohnheim states that 8 c.c. pancreas means the alcoholic extract of 0·8 gramme of fresh pancreas; it is scarcely conceivable that 10 c.c. of blood serum should contain the same amount of activating body as 1 gramme of the gland that secretes it.

A further point that invites criticism in the above experiment is the fact that the muscle alone gave no glycolysis at all. Now it is scarcely possible even in an animal killed by bleeding that the tissues should be so freed of blood and serum that 100 grammes of muscle (amount said to be used in these experiments) should not contain, say, 2 c.c. of serum. If so, and if 7 c.c. is the optimum value, then surely the muscle itself should show some considerable glycolytic power if the serum used was, as seems probable, taken from the same cat.

In the next experiments I attempted to imitate exactly the method followed by Cohnheim. The muscle was obtained from rabbits and cats killed by bleeding, by as rapid and aseptic a dissection as was possible; it was passed, after weighing, through a mincing machine into ice cold water; sugar, about 1 gramme per 100 grammes muscle, 10 per cent. toluol and 2 per cent. magnesium carbonate (to preserve neutrality, instead of the solution of mixed phosphates previously used) added, and the whole made up to nearly the required volume with water and ice. After standing for six to twenty-four hours at 0° C., the remaining ice was removed, and the volume of the fluid again adjusted. The mass was then well shaken and filtered through sterilised cloth and separated into equal portions. The minced pancreas of the same animal was used in some cases, in the others the mixed pancreatic extract previously prepared. Determinations were made in some cases by estimating with Pavy's solution in the presence of protein; in other cases after removal of the protein. Average dilution to five or ten times the volume. After the control determination the flasks were sealed with cotton wool and incubated twenty hours and upwards at 37° C., and then re-estimated.

To remove the protein 40 c.c. of the fluid was measured, neutralised by acetic acid, and then a slight excess of acetic acid and about 10 grammes of magnesium sulphate added; the whole diluted to 100 c.c. and heated for twenty minutes on a water bath. The precipitate was broken up finely by a glass rod, and the whole removed by washing to a measuring cylinder and made up to 40 c.c. or other required volume and allowed to stand twenty-four hours, then filtered and used in the estimation. In

earlier experiments I had found that even prolonged washing did not extract all the sugar from the protein precipitate, but in this way the results obtained gave only a slighter lower value than direct estimations with Pavy before freeing from protein.

SERIES C.—Fresh muscle minced and extracted with ice cold water in the presence of dextrose, magnesium carbonate, and toluol.

Experiment XI.—Cat's muscle; extracted 6 hours at 0°, filtered. 200 c.c. = 100 grms. muscle. Minced fresh pancreas of same cat. Estimated by Pavy direct at dilution of 10 volumes.

	Reducing power per portion in grms. dextrose		Difference in reducing power as grms. sugar		
	Before	After 24 hrs.	Per experiment	Per cent.	Per kilo. muscle
a 200 c.c. (100 grms. muscle)	0·980	0·971	−0·009	−1	−0·09
b 200 c.c. + 1 grm. pancreas	0·980	0·909	−0·071	−7	−0·71
c 200 c.c. + 0·3 grm. pancreas	0·980	0·961	−0·019	−2	−0·19

Reactions alkaline after incubation toluol abundant. Smell sweet. Cultures gave no growth.

Experiment XII.—Cat's muscle: extracted 6 hours at 0°, filtered. 200 c.c. = 100 grms. muscle. Minced fresh pancreas of same cat. Estimated after removal of protein.

	Reducing power per portion in grms. dextrose		Difference in reducing power as grms. sugar		
	Before	After 24 hrs.	Per experiment	Per cent.	Per kilo. muscle
a 200 c.c.	0·962	0·962	0·000	0	0·00
b 200 c.c. + 1 grm. pancreas	0·926	0·909	−0·017	−2	−0·17
c 200 c.c. + 3 grms. pancreas	0·961	0·971	−0·014	−1·5	−0·14

Experiment XIII.—Fresh minced Cat's muscle extracted as above. Mixed alcoholic pancreatic extract used. Estimated by Pavy in presence of protein. Incubation 20 hours.

	Reducing power per Experiment in grms. dextrose		Difference in grms. Dextrose		
	Before	After 20 hrs.	Per experiment	Per cent.	Per kilo. muscle
a 200 c.c. = 100 grms. muscle	0·980	0·971	−0·009	−1	−0·09
b 200 c.c. + 20 c.c. PE(iii)	0·980	1·026	+0·047	+5	+0·47
c 200 c.c. + 10 c.c. PE(iii)	0·980	0·931	−0·049	−5	−0·49
d 200 c.c. + 1 c.c. PE(iii)	0·980	0·956	−0·024	−2·5	−0·24

All alkaline and sweet after 24 hours; excess of toluol.

Experiment XIV.—Fresh cat's muscle extracted six hours at 0°. Mixed pancreatic extract. Estimated after removal of protein. Incubation 24 hours.

	Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
a 200 c.c. = 100 grms. muscle	0·962	0·962	0	0	0
b 200 c.c. + 20 c.c. PE (iii)	0·981	0·982	+0·001	0	+0·01
c 200 c.c. + 10 c.c. PE (iii)	0·982	0·932	−0·050	−6	−0·5
d 200 c.c. + 1 c.c. PE (iii)	0·990	0·971	−0·019	−2	−0·2

All alkaline and sweet after 24 hours; excess of toluol.

Experiment XV.—Fresh cat's muscle extracted at 0° for six hours. Fresh cat's pancreas. Incubated 48 hours. Pavy in presence of protein.

		Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	200 c.c. = 100 grms. muscle	0·980	0·980	0	0	0
b	200 c.c. + 1 grm. pancreas	0·980	0·918	−0·062	−7·3	−0·72
c	200 c.c. + 0·3 grm. pancreas	0·980	0·971	−0·009	−1	−0·09

All alkaline and sweet after incubation; excess of toluol present.

Experiment XVI.—As Experiment XV, but pancreatic extract used. Incubation 48 hours.

		Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	200 c.c. = 100 grms. muscle	0·980	0·980	0	0	0
b	200 c.c. + 20 c.c. PE(iii)	0·980	1·009	+0·029	+3	+0·29
c	200 c.c. + 10 c.c. PE(iii)	0·980	0·972	−0·008	−1	−0·08
d	200 c.c. + 1 c.c. PE(iii)	0·980	0·976	−0·004	−0·5	−0·04

All alkaline and sweet after incubation; excess of toluol present.

Experiments XVII and XVIII.—Cat's muscle extracted as above for six hours with ice cold water, and filtered. Residue again extracted for 24 hours with ice cold water with sugar, etc., and filtered. Extract such that 150 c.c. = 100 grms. muscle. Alcoholic pancreatic extract used. Incubation 24 hours.

Experiment XVII.—Estimations in presence of protein.

	Extract	Reducing power per experiment in grms. dextrose		Difference in reducing power in grms. dextrose		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	95 c.c. in 100 c.c.	0·143	0·150	+0·007	+5	+0·11
b	90 c.c. + 2 c.c. PE (iii) in 100 c.c. ...	0·135	0·130	−0·005	−3	−0·09
c	95 c.c. + 1 c.c. PE (iii) in 100 c.c. ...	0·143	0·143	+0·0	+0	0
d	95 c.c. + 1 c.c. PE (iii) + AmCarb in 100 c.c.	0·143	0·148	+0·005	+3	+0·08
e	90 c.c. + 5 c.c. PE (iii) in 100 c.c. ...	0·135	0·148	+0·013	+9	+0·22
f	45 c.c. + 30 c.c. PE (iii) in 100 c.c. ...	0·068	0·076	+0·008	+2	+0·26
g	95 c.c. + 5 drops PE (iii) in 100 c.c. ...	0·143	0·139	−0·004	−3	−0·06

All alkaline and sweet after 24 hours; excess of toluol present.

Experiment XVIII.—Estimations after removal of protein.

		Reducing power per experiment in grms. dextrose		Difference in reducing power in grms. dextrose		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	95 c.c. in 100 c.c.	0·140	0·148	+0·008	+5	+0·12
b	90 c.c. + 2 c.c. PE (iii) in 100 c.c. ...	0·133	0·136	+0·003	+2	+0·05
c	95 c.c. + 1 c.c. PE (iii) in 100 c.c. ...	0·140	0·140	0	0	0
d	95 c.c. + 1 c.c. PE (iii) + $AmCl_3$ in 100 c.c.	0·140	0·137	−0·003	−2	−0·05
e	90 c.c. + 5 c.c. PE (iii) in 100 c.c. ...	0·133	0·139	+0·006	+4	+0·10
f	45 c.c. + 30 c.c. PE (iii) in 100 c.c. ...	0·066	0·075	+0.009	+6	+0·30 gm.
g	95 c.c. + 5 drops PE (iii) in 100 c.c. ...	0·140	0·137	−0·003	−2	−0·05 gm.

All alkaline and sweet after 24 hours; excess of toluol present.

Experiment XIX.—Minced fresh rabbit's muscle, extracted 24 hours at 0° C. in presence of sugar, mag.-carb. and thymol. Filtered through cloth and incubated 24 hours at 37° C. 200 c.c. = 100 grms. muscle.

		Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	40 c.c. extract of muscle	0·746	0·726	−0·02	2·7	−1 gm.
b	40 c.c. muscle extract + 20 c.c. PE (iii)	0·746	0·737	−0·01	1·3	−0·5 gm.

Apparently quite sweet. Reaction remained alkaline.

Experiment XX.—As above, but extracted 72 hours. Incubated 24 hours.

		Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	30 c.c. extract	0·054	0·008	−0·046	−90	−3·1 gm.
b	30 c.c. extract + 3 c.c. PE (iii) ...	0·054	0·057	+0·003	+ 6	+0·2 gm.
c	30 c.c. extract + 6 c.c. PE (iii) ...	0·054	0·053	−0·001	− 2	−0·06 gm.

a Contained mould and bacilli in abundance. Reaction acid.

b and *c* Apparently sweet. Reactions alkaline.

Experiment XXI.—As Experiment XIX, extracted 24 hours; incubated 48 hours.

		Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
		Before	After	Per experiment	Per cent.	Per kilo. muscle
a	40 c.c. extract	0·727	0·708	−0·019	−2·6	−0·95 gm.
b	40 c.c. extract + 20 c.c. PE (iii) ...	0·727	0·729	+0·002	+0·3	+0·1 gm.

Reactions alkaline, apparently quite sweet.

Comments on Series C

Experiments XI to XVI. In these experiments the muscle extract alone had practically no power of glycolysis whatever. In the presence of fresh pancreas a slight glycolysis was seen, never amounting to more than 0·72 gramme per kilogramme muscle, that is to 10 grammes of sugar or 7 per cent. of the total sugar used; an amount, which, considering the elaborate technique and the numerous chances of error from evaporation of toluol from large dilution, etc., seems to approach too nearly to the limits of experimental error to be of any value.

Experiments XVII and XVIII form two exactly parallel series; the production of reducing substance by large amounts of pancreatic extract is again seen, but small amounts led to extremely little glycolysis, never in amount which could possibly be sufficient to account for the mechanism of the metabolism of carbohydrate in the body. Experiments XIX to XXI are again negative.

On further consideration of Cohnheim's articles, the fact again suggested itself that of all his experiments he obtained the most regular and striking results in those cases where the muscle and pancreas of the animal were mixed intimately from the first by being minced together, and in line with this was the fact that in the first series of experiments, where somewhat similar conditions obtained, my results had approached his most nearly.

Now if the muscle contains some substance, ferment or complement, which can, when influenced by the activator or amboceptor contained in the pancreas, cause glycolysis, this body must be extremely liable to be destroyed by slight changes in its environment, possibly susceptible to the autolytic ferments of muscle itself. If so, it is possible that just as other ferments are protected from destruction by the presence of the substances on which they exert their action, so this muscle ferment might be protected from destruction and obtained in greater amount and of higher activity if protected from the outset by the presence not of sugar (as obtained in Series C), for it does not on analogy combine directly with that substance, but of its pancreatic complement, and possibly even better protected by the presence of sugar also. This would explain the greater regularity of results obtained as described above by Cohnheim in his first contribution.

I had hoped that by some modification of the technique of the workers on protection and immunity that I might further be able to isolate this muscle ferment either alone or combined with its amboceptor

from the muscle proteids, and so get a more striking demonstration of its existence, but their work as yet holds out no hopes of such success.

However, fresh muscle was extracted in ice cold water for twenty-four and seventy-two hours in the presence of pancreatic extract in varying amounts, sugar, magnesium carbonate and thymol (toluol not being, unfortunately, at the time obtainable). Unfortunately the thymol did not prove as successful an antiseptic as toluol had done, and a large proportion of the flasks were found after incubation to be very foul and to contain mould and numerous other organisms. Those in which this was obviously present were not estimated, nor those in which the smell was other than sweet or in which the contents of the flask had not the usual appearance of a clear slightly reddish fluid, alkaline in reaction, with a loose flocculent deposit.

SERIES D.—Minced fresh rabbits' muscle extracted with ice cold water in the presence of pancreatic extract, sugar, and mag.-carb. Thymol as disinfectant in great excess (most flasks smelt strongly of thymol). Filtered through cloth after extraction. Fluids estimated before and after incubation with Pavy in the presence of protein. 300 c.c. extract equivalent to 100 grms. muscle.

Experiment XXII.—Controls. 24 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
200 c.c. muscle extract without pancreas = 66 grms. muscle	0·363	0·354	0·007	−2	0·105
		Alkaline, sweet.			

N.B.—72-hour control obviously contaminated.

Experiment XXIII.—24 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 4 c.c. PE (iii)	0·368	0·338	0·030	− 8	0·450

Though alkaline and apparently sweet, mould and bacillus (? Hay bacillus) present in considerable amount.

Experiment XXIV.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 10 c.c. PE (iii)	0·391	0·095	0·296	— 75	4·44

Mould and bacillus found in scanty amount microscopically. Reaction alkaline, smell not quite sweet, cultures lost.

Experiment XXV.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
200 c.c. muscle extract (= 166 grms. muscle + 10 c.c. PE (iii)) and 16 c.c. PE (iii) added just before incubation ...	0·389	0·341	0·048	−15	0·72

Apparently sweet. Residue after 72 hours obviously foul.

Experiment XXVI.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 4 c.c. PE (iii)	0·363	0·320	0·043	−14	0·65

Apparently sweet. Residue in 72 hours still sweet.

Experiment XXVII.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per experiment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 6 c.c. PE (iii)	0·376	0·372	0·004	− 1	0·06

Alkaline, remained sweet after 72 hours.

Comments on Series D

Here Experiments XXVII, and possibly XXVI, alone could be relied on, and these are negative or small; but the series is put in at length as showing the varied action of the bacteria and the way in which their growth is favoured by pancreatic digestion of the medium.

Conclusions

1. That the glycolysis obtained by the combined action of pancreatic and muscle juices is not as a rule greater than the sum of the actions of its constituents.

2. That slight errors in technique tend to cause the appearance of such an increase, owing to bacterial growth being favoured by pancreatic digestion.

3. That such bacterial action may be masked at first when large amounts of pancreatic juice or extract are used by the appearance of protein breakdown products with reducing powers.

4. That the difficulty of maintaining asepsis in such a series of observations is almost insuperable without special apparatus, and that while antiseptics such as toluol retard, they do not prevent absolutely the action of micro-organisms in the solutions.

5. That in such a series of experiments the extreme difficulty of estimating sugar in the presence of protein leads to a high degree of experimental error; that removal of the protein before estimating only overcomes this by introducing a further series of errors, and the figures are quite unreliable for small changes in concentration in solutions originally containing from 2 to 5 grammes of dextrose per litre.

6. That by incubation of fresh muscle juice or extract, especially in the presence of pancreatic juice or extract, large amounts of reducing substance are often formed, possibly from glycogen in some degree, but more largely from autolytic or tryptic digestion of the muscle plasma, and that the nature of these bodies might well repay detailed investigation.

7. That, in face of these facts, it is useless to attempt to find any explanation, along the lines of the present research, for the failure of pancreatic extracts to benefit cases of diabetes.

I take this opportunity of expressing my thanks to Dr. H. MacLean and Professor Benjamin Moore for their advice and assistance in the course of these experiments.

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ON THE EXISTENCE OF A REDUCING ENDO-ENZYME IN LIVER AND KIDNEY

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Historical

Hoppe-Seyler[13] was the first to draw attention to strong reducing processes in living tissues. He suggested that by means of reduction molecular oxygen was rendered active by conversion into nascent oxygen and thus oxidised tissue somewhat after the manner of hydrogen-saturated palladium-foil which can oxidise indigo.

Paul Ehrlich[5] two years later, in 1885, published his researches on the reducing-power of tissues *intra vitam* and at the moment of death. He classified tissues as regards their oxygen-avidity as follows:—

1. Those in which indo-phenol-blue remained unchanged; these he regarded as saturated with oxygen. Examples: heart, renal cortex and the grey matter of the central nervous system.

2. Those which reduce indo-phenol-blue to indo-phenol-white, but not alizarine blue to alizarine white. Examples: striated and non-striated muscle, gland-parenchyma.

3. Those which reduce alizarine blue to alizarine white, those with greatest oxygen avidity. Examples: lung, liver, fat-cells and the gastric mucosa.

Ehrlich injected the pigments subcutaneously *intra vitam*. He pointed out that in the case of naturally *red* organs—e.g., heart and spleen—it is difficult to appreciate degrees of blueness, so he plunged them into hot water; this makes them paler. Ehrlich noticed that heat arrested the reducing power. He did not suggest that tissue-reduction was due to an enzyme.

J. de Rey-Phailhade[25, 26] wrote extensively between 1888 and 1909 on a substance which he called 'philothion,' which he regarded as one of the mercaptans and indistinguishable from cystëine. To this substance he attributed extreme importance in the fixation of oxygen by the tissues,

He thus represented the reactions involved:—

$$R\begin{matrix}S\cdot H\\S\cdot H\end{matrix} + O = R\begin{matrix}S\\|\\S\end{matrix} + H_2O \text{ and}$$

$$R\begin{matrix}S\\|\\S\end{matrix} + 2\,(H\cdot OH) + M = R\begin{matrix}S\cdot H\\S\cdot H\end{matrix} + M\cdot(OH)_2$$

'M etant un corps capable de fixer 2 (OH).'

Rey-Pailhade further remarks:—'L'hydrogene philothionique que se transporte façilement sur certaines matières colorantes explique clairement les phénomenès de réduction observés par Ehrlich *in vivo*.'

Spitzer[27] noticed that for a time after the death of the animal, while the reducing-power of the tissues increased, the oxidising capacity rapidly disappeared. He also noticed that the temperature of 100° C. might not always destroy the reducing-power, whereas it always destroyed the oxidising.

Victor Horsley and A. Butler Harris[14] made a report to the Scientific Grants Committee of the British Medical Association on the appearance of tissue of animals injected subcutaneously *intra vitam* with methylene blue. The gastric mucosa at first secreted it blue, later greenish. In the milk and in the urine a leuco form was found. In the saliva, at first neither the blue nor the leuco form was found, later the blue appeared.

On faradisation of the living *cortex cerebri* these workers found a state of reduction at the stimulated spot at a time when the blue coloration was at its height. The resting warm tissue became blue. The decoloration was not due to ionised hydrogen at the kathode, for when the cortical excitability had disappeared the reduction of the pigment could no longer be obtained.

Frequently they found that oxidation prevailed over reduction; thus the pylorus was blue when active digestion was in progress, and the voluntary muscles were very blue whilst active, maximal oxidation being thereby indicated. Muscles with cut nerves showed a more intense blue than did normal muscles, minimal reduction and minimal oxidation being indicated as simultaneously existing. In 1896 I[6, 7] found that living tissues from the cat and rabbit—such as kidney, liver, heart, the glands—reduced the blue potassio-ferric-ferrocyanide in the Prussian blue and gelatine injection-mixture to the green or white (leuco) state of the di-potassio-ferrous-ferrocyanide, which on exposure to the air or by treatment with hydrogen peroxide at once became blue again. The pigment was reduced only in the washed-out smaller vessels and capillaries; in presence of blood not washed out of the larger vessels the Prussian blue remained unreduced.

The term 'reductase' as indicating a tissue-ferment capable of effecting reduction-processes was first used by Abelous and Gerard[1] in 1899.

Pozzi-Escot[23] published the results of work on the reducing action of vegetable and animal tissues on solutions of indigo, litmus and Prussian blue out of contact with the air. He held that a reductase may be suspected when living tissue decomposes hydrogen peroxide but does not affect a mixture of guaiacum and hydrogen peroxide. He confirmed Rey-Pailhade in finding that tissues could form di-hydrogen sulphide from sulphur, and could reduce potassium iodide when out of contact with the air. Oxidases, he said, are to be recognised by the blue coloration with guaiacum and by the fact that they re-oxidise phenol-phthalin to phenol-phthalëin 'so easily recognised by alkalies.'

C. A. Herter[15, 16] published two papers on the reducing powers of the living tissues. He used Grübler's methylene blue dissolved in 0·85 per cent. sodium chloride and injected it into the jugular vein of the rabbit *intra vitam* under chloroform anaesthesia. He stated that 'the liver usually retains a high grade of reducing activity for several hours after death.'

During life the lungs, supra-renals and grey matter reduce methylene blue to the 'leuco base.' These are more powerful reducers than muscles, spleen, kidneys and connective-tissue. An animal which was chilled by wet cloths or by ice 'exhibited the power of reduction much diminished by cold.'

Herter showed that, on the other hand, increased tissue metabolism produced by experimentally induced fever (hog-cholera) was accompanied by a greatly increased reducing action towards methylene blue and indophenol. He found the post-mortem decline of reducing power in rabbit's muscle was very marked. He believed chloroform retarded the rate of reduction.[18].

F. P. Underhill and O. E. Closson[29] published the results of a research into the behaviour of methylene blue and methylene azure in the living organism. Their paper, which is mainly of chemical interest, confirms Herter in that 'the experiments demonstrate the simultaneous action of both oxidation and reduction processes in the animal organism.'

In conjunction with J. C. Irvine, of the Chemical Department, the University, St. Andrews, I[9] showed that the *intra vitam* reduction of Prussian blue was not a deoxidation but the removal of an ionic charge. We insisted that the reduction of methylene blue to methylene white was

not a deoxidation, in spite of the statements of several authors, recently, for instance, that of Hill in 'The Body at Work'[12]:—'Methylene blue contains some easily removable oxygen.'

By perfusing the surviving washed-out kidney of the sheep with the Prussian blue mixture, I obtained from the cannulated ureter an absolutely colourless, gelatinous, artificial 'urine,' which was blued immediately on the addition of hydrogen peroxide. At the time these experiments were done I had not seen any of the papers by Herter or his pupils.

A. Heffter[10, 11] has recently published papers on work done on the reducing power of tissue-extracts. He opposes the notion that the existence of a reductase is needed to carry out any of the reductions performed by living tissues or tissue-juices. He holds that the labile hydrogen of colloids in such a grouping as cystëine is able to effect all the reductions observed. Autoxidation is the more important phenomenon of tissue vitality, reduction an unavoidable concomitant. Crystallised egg-albumin can bring about many reductions.

G. P. Mudge[22] has made a communication on the pigmentation of hairs as due to the interaction of a chromogen and a ferment. He thinks that the albino, for instance, has only the chromogen. Regarding fermentation as a process either of oxidation or reduction he thus writes: 'If an albino does carry a chromogenous body which only needs the influence of an oxidising or reducing ferment to cause it to produce pigment,' etc.; that is to say, this author recognises the functional existence of a 'reductase.'

Experimental Methods

Preparation of the press-juices. I employed press-juice from perfectly fresh livers of the sheep and frog and from kidneys of the ox.

A Klein's press for tissue-juice was used.

The following will indicate the typical procedure followed to obtain fairly large quantities of the juice:—

The entire liver was without delay perfused with 0·9 per cent. sodium chloride solution in tap-water at a temperature of 40° C. through the portal vein until the fluid from the hepatic vein was bloodless. The mass was then allowed to drip suspended in a piece of muslin previously washed free from starch and was then cut up into small pieces which were pressed over thick flannel in the Klein's press. From a liver weighing about 1,000 grammes, 200 c.c. of press-juice could be obtained by this method,

The procedure for the ox kidney was similar.

The keeping of the juice. Obviously it is of the utmost importance to prevent the press-juice decomposing, as even traces of 'products of putrefaction' have intense reducing powers. Toluene and benzene were the only antiseptics I used. Battelli[2] has emphasised the inhibitory effect of antiseptics on the enzymic and respiratory powers of tissues. It may be assumed that antiseptics do, to some extent, inhibit enzymic action. Fresh juice well shaken with toluene and left under a layer of toluene will remain perfectly undecomposed and active for upwards of six weeks; but there is a progressive weakening of reducing power, until at the end of three months the juice is quite inoperative.

In all routine work I purposely refrained from using any saline or acid substance in case such might prove to be an inhibitant or an activator of the enzyme investigated. Sodium fluoride, for instance, I rejected on these grounds.

It is clear that the reductions observed in the following instances were not due to putrefactive or autolytic products, for had that been so, the juice would have acquired more and more reducing-power instead of less as time went on. After six weeks there is a falling off in the energy of reduction, and by ten to twelve weeks it has practically disappeared.

Hepatic juice three months under toluene was pronounced sterile after bacteriological examination by Dr. Sholto Douglas, to whom my thanks are due for having examined the material. At his suggestion I kept all juice expressed after December, 1909, in paraffin-lined flasks, as the alkalinity of glass is known to cause the activity of certain enzymes to diminish.

Pigments used in judging of reduction. Unless when otherwise stated, I judged of reduction, and therefore presumably of the presence of a reductase, by the change of colour or fading of the following three solutions kept at 41° C., to which press-juice had been added:—

1. Potasio-ferric-ferrocyanide (soluble Prussian blue) of 0·062 per cent. strength in distilled water.

2. Sodium-indigo-disulphonate (indigo blue) of 0·08 per cent. strength in distilled water.

3. Methylene blue of 0·0125 per cent. strength in distilled water.

Usually 10 c.c. of one or other of these solutions were mixed with 3 to 5 c.c. of the juice being investigated.

The above concentrations were arrived at after a number of trials to discover the most suitable strength for the coloured solutions, so that they would show within a few hours some degree of fading or change of colour in presence of 3 to 5 c.c. of juice. *Quite fresh* hepatic or renal juice will cause Prussian blue to fade within a minute or two at room temperature.

Certain characters of the press-juice. Examined under the microscope, no intact cells could be seen in either hepatic or renal juice. The juice was a highly granular, pinkish, opaque liquid filterable with extreme slowness through flannel; not filterable through paper.

It had at first a neutral reaction, but liver juice acquired an acid reaction (to litmus) on being kept for a few days.

On boiling, a massive heat-coagulum was obtained.

By saturating with magnesium sulphate it was possible to separate a cell-globulin from a cell-albumin.

The first thing to ascertain was whether 'raw' liver press-juice reduced any pigment to the leuco condition.

Five c.c. of the fresh liver juice were shaken up with toluene and added to 10 c.c. of dilute solutions—at that time of unknown strengths—of soluble Prusian blue, sodium indigo-disulphonate and methylene blue, respectively, and were thereafter placed in the thermostat at 41° C. A control in each case consisted of the filtrate from an equal volume of juice boiled for five minutes. In four hours the Prussian blue was seen to be of a very pale green colour, which was immediately restored to a deep blue by the addition of hydrogen peroxide. The indigo blue was entirely bleached within twenty-four hours, the methylene blue within the same time was rendered considerably paler. The filtrate from the heat-coagulated juice failed in each case to effect any degree of reduction whatever. The inference seemed justified that we were here dealing with an endo-enzyme not indeed isolated but dissevered from the intact hepatic cells and associated with the cell-proteins. It seemed capable (1) of reducing a pigment that contained oxygen, the indigo blue, (2) of reducing pigments that contained no oxygen (Prussian blue and methylene blue), and (3) it was destroyed at the temperature of 100° C.

The criticism at once occurred to me; might this change of colour not be 'bleaching due to the true presence of organic material.' If, for instance, white-of-egg be mixed with Prussian blue, the colour is discharged within twenty-four hours, and in a less degree indigo blue is bleached by egg-white. Similarly potassium permanganate is 'reduced'

to a colourless condition by organic matter presumably containing no enzyme whatever.

I therefore used for the experiment on this point fresh liver-juice in presence of toluene, and for the control an equal amount of heat-coagulated liver-juice (100° C.). Thus if the bleaching were due to the presence of organic matter, the control tubes ought to have shown as much or nearly as much reduction as the tubes with the unaltered juice. The result was that the tubes containing the boiled juice were scarcely affected. The mere presence of organic material was therefore not the active agent in bringing about the reduction, but this was presumably due to an endo-enzyme destroyed at the temperature of 100° C.

On the spontaneous re-blueing of the chromogen. At an early stage I noticed that in a tube in which the Prussian blue had been completely reduced to the leuco state, a re-establishing of the colour was evident from about the third or fourth day onwards. A mixture of fresh liver-juice shaken up with pigment of suitable strength would begin to become blue again, always beginning at the top, in spite of the fact that the mixture was covered by a layer of the lighter toluene to the depth of an inch. This I provisionally explained by assuming that the restoration of colour was due to the activity of an oxidase utilising atmospheric oxygen which had diffused through the supernatant toluene. Later I got quite independent evidence of the presence of an oxidase in the press-juice of both liver and kidney. To test this point, I arranged to keep fresh liver-juice in toluene in presence of some Prussian blue under an atmosphere of pure hydrogen, with the object of seeing whether one might thus permanently avoid atmospheric oxidation. The experiment was quite successful; the pigment remained in the leuco state for three and a half months. On breaking open the vessel and adding hydrogen peroxide, the contents immediately became bright blue. Exposure to the air produced the same result but more slowly.

Oxidative restoration of the pigment by a co-existing oxidase is therefore a factor to be borne in mind in these experiments on endo-enzymic reduction.

The Variations of Reducing Activity related to Variations of Temperature

These are characteristic of enzymic action and are well shown in the case of hepatic or renal reductase.

A typical experiment may be quoted:—

Three water-baths were brought to (*a*) between 40° C. and 41° C., (*b*) 42° C. and 43° C., and (*c*) 44° C. and 46° C. respectively. In each a tube was placed containing 3 c.c. of raw hepatic juice shaken up with 12 c.c. of Prussian blue under toluene. In six hours the tube in (*a*) was blue-green, that in (*b*) was white-green, the one in (*c*) was quite white; twenty-four hours later the tube in (*b*) was white. I frequently observed the acceleration in bleaching at 40°C. as compared with room-temperature (15° C.). The inference here is that we are dealing with an endo-enzyme the velocity of whose action is distinctly accelerated by a rise of temperature from 15° C. to 46° C.

Herter, using 'triturated tissues' in presence of an aqueous solution of methylene blue, found a marked acceleration of reduction at 43° C.

The effects of cold. Taking the degree of reduction exhibited in a tube containing liver-juice and Prussian blue at 15° C. as the standard, I compared this with a similar mixture kept in an ice and salt freezing mixture for several hours. The contrast was very marked; there was no appearance of fading in any of the chilled tubes at temperatures varying from zero to — 14° C.

This marked inhibition by cold corroborates Herter's observation with methylene blue in the intact animal, 'the power of reduction . . . . is much diminished by cold,' and 'a considerable fall in body-temperature is attended by a diminished reduction of methylene blue to leuco methylene blue, and this result is particularly striking and unequivocal in the case of the muscles including the heart and diaphragm and the grey substance of the central nervous system.'

Attempts to Determine the 'Death' Temperature

The determination of the exact temperature at which reductase ceases to act is for many reasons a very difficult matter.

The volume of juice taken is a factor to be reckoned with. I took as standard the behaviour of 5 c.c. of raw hepatic juice kept in contact with 12 c.c. of Prussian blue for twenty-four hours at 41° C. In order to judge of the colour of the pigment unobscured by the presence of precipitates, heat-coagula, etc., the contents of the tubes were filtered and the depth of colour of the filtrate carefully scrutinised. A series of

these tubes was kept under observation, thus:—

(*a*) for twenty-four hours at 41° C.,

(*b*) for twenty-four hours at 70° C. to 73° C.,

(*c*) for twenty-four hours at 80° C. to 83° C.

On filtering off the liquid present in each case, that from (*a*) was seen to be of a very pale green, that from (*b*) was blue-green, while that from (*c*) was almost a pure blue colour.

I had evidence that above 60° C. there was progressive inhibition of the energy of reduction. The destruction-temperature seems to lie between 75° C. and 80° C. Juice heated to temperatures above 80° C. did not adsorb the pigment so thoroughly as juice less highly heated. The general inference to be drawn from a large number of observations on the effect of heat on the enzyme is that the cell-proteins protect the reductase from the destroying effects of heat.

Efforts to obtain the Enzyme in solution

The typical method of extraction with alcohol was first carried out with a view to induce the ferment to pass into solution in distilled water. The results of this procedure were entirely negative. The liquid contained protein and yielded flocculi at 41° C., but it possessed no reducing power. The inference was that the alcohol had either destroyed the reductase in the raw juice or had rendered it insoluble in water, if it was originally soluble. To test the point as to possibility of the alcohol having destroyed the reductase, I omitted alcohol in preparing the aqueous extract thus—Some fresh liver juice under toluene was dried in vacuo at 17° C., while an equal quantity was dried at 100° C. over the water-bath. Both portions were extracted by prolonged grinding with sand in distilled water. To a series of the three pigments a few cubic centimetres of the vacuo-dried extract were added and the tubes kept for eighteen hours at 41° C. A control series was made with the extract of the material dried at 100° C. In no tube of the six was there any reduction whatever. The inference here is that drying the fresh juice in vacuo at room temperature (and a fortiori at 100° C.) destroys the reductase and renders it insoluble in water or otherwise inactivates it. That it is destroyed is probable from the further fact that subsequent addition of some of the powdered vacuo-dried juice caused no reduction in any of the tubes within so long a time as three to four days.

Professor Adrian Brown kindly suggested to me the possibility of the endo-enzyme being originally insoluble, but capable of becoming

active on its being moistened in its inhibited condition in vacuo-dried juice. Accordingly I moistened some vacuo-dried liver juice with some distilled water and added the paste to Prussian blue kept at 40° C. There was no reduction until putrefaction set in. The inference seems to be that whether the reductase is originally soluble or insoluble, it is rendered profoundly inactive by desiccation even at ordinary temperatures.

The following conditions then seemed possible:—Either the reductase existed in the liver-cells and was subsequently in the press-juice in an insoluble state, but capable of reducing pigments in solution (treatment with alcohol or desiccation being highly injurious to it), or the reductase was originally soluble but so sensitive to alcohol or desiccation at any temperature—ordinary, medium or high—that it was rendered insoluble or inhibited or destroyed.

Proceeding on the assumption that the process of drying was injurious to the ferment owing to its being in some artificial condition in press-juice as compared with its natural state in intact hepatic cells, I dried small pieces of perfectly fresh liver on the water-bath at 40° C., while an equal weight was simultaneously dried at 100° C. Watery extracts of neither of these substances effected any reduction. The inference from these observations seemed to be that the reductase was originally insoluble in the liver cells and non-extractable by water or so bound to the cell-proteins that desiccation at a temperature of 41° C. rendered it insoluble or non-extractable by water. Accepting then the position that treatment with alcohol or by desiccation was equally inadmissible, I proceeded to study the behaviour of a relatively purer form of liver-juice than the raw juice as it comes from the tissue-press.

A sheep's liver was perfused as usual until bloodless, allowed to drip, pressed through flannel, and the juice thoroughly ground up in a mortar with fine sand and toluene. The fluid was then centrifugalised and filtered through two layers of a Schleicher and Schüll's filter-paper. A non-granular liquid came through with extreme slowness. A little of this liquid added to the pigments and kept at 41° C. caused them to assume a curious and characteristic appearance which I have since noticed on other occasions; I call it 'the white and blue appearance.' The whole interior of the tube is lined by the flocculent precipitate which has adsorbed the whole of the pigment, but on the side towards the lumen of the tube the precipitate is white, on the side next the glass it is blue. The inference from this seems to be that where the juice is in free contact with the maximum of water, there the reductase is under the maximum condition

for its activity, where the water is at a minimum the ferment is inactive. The reductase seems to cling to the cell-proteins and to be entangled and adsorbed by them when they are precipitated at 41° C.

To the extent that the proteins are soluble in normal saline the reductase is soluble along with them, but so far, I have not succeeded in obtaining a solution of the endo-enzyme apart from the cell-proteins.

Still avoiding both alcohol and drying, I wished to submit the press-juice to exceedingly thorough stirring with normal saline to induce the endo-enzyme to pass into solution if that was by any means possible. Two hundred c.c. of hepatic juice (sheep) were mixed with 200 c.c. of 0·75 per cent. sodium chloride and some toluene, and the whole was stirred by a glass stirrer driven by a water-motor for sixty hours. The stirred liquid was then filtered through a layer of paper over a porous porcelain vessel by an exhaustion-pump. The filtrate, which was yielded very slowly, was a rather viscous, clear brown liquid with the following characters:— It gave the xanthoproteic reaction at once; on saturation with magnesium sulphate a precipitate of cell-globulin was obtained; it reduced Fehling's solution very slightly; it gave no blue with guaiacum alone nor with hydrogen peroxide subsequently added. I allude to it as the 'exhaust filtrate.' Five c.c. of the exhaust filtrate gave with Prussian blue at 41° C. in a few hours the white and blue appearance. A similar mixture kept at air temperature presented the same appearance after a longer interval. The inference again is that the reductase was in intimate association with the cell-proteins which adsorbed the pigment.

I next tried to discover whether the ferment was associated with one cell-protein more than with another. On saturating with ammonium sulphate and filtering, the filtrate did not reduce Prussian blue at all, whereas the precipitated material did, the inference being that the reductase was entangled with all the proteins. This was corroborated by finding that on saturation with magnesium sulphate both the filtrate and the original saline precipitate reduced Prussian blue (with the white and blue appearance).

Thus the endo-enzyme seems to be able to associate itself either with the globulins or with certain other cell-proteins, but not to be able to free itself from some one or more of these cell-constituents. The degree of reduction as compared with that effected by the raw juice was less intense in the case of the various preparations referred to—filtrates, exhaust juice and saline precipitates and filtrates therefrom.

Attempt to obtain the Endo-enzyme in solution by dialysis

These laborious and numerous experiments were negative. I completely failed by dialysis through parchment tubes in tap water to induce any ferment to enter the water outside.

The inference from this is confirmatory of the finding as to the insolubility of reductase. This insolubility is in striking contrast with the high solubility in water of the catalase separated by Usher and Priestley[30] from the sheep's liver. A catalase capable of liberating oxygen from hydrogen peroxide was obtained from dried liver powdered and extracted with water, the solution being precipitated with excess of absolute alcohol. This catalase as compared with hepatic reductase is either very stable or insensitive to physico-chemical reagents.

Reduction with Substances other than the Three Blue Pigments

Fehling's solution is slowly but slightly reduced, as is also osmium peroxide: neither is a suitable substance for routine work.

To the use of ferric thiocyanate, ferric chloride, ferric sulphate and potassium permanganate there are objections of various kinds. They have all been used, but have not yielded results worthy of being recorded. As to organic substances: alkaline pyrogallol is unsuitable owing to the speed with which it becomes reoxidised in contact with the atmosphere.

Quinone. By both liver and kidney juice this substance is reduced to quin-hydrone, although not to the colourless hydroquinone. Probably the simultaneous presence of the oxidase prevented this.

I could obtain no evidence that either hepatic or renal juice could reduce sulphur to hydrogen sulphide after the manner of a ferment produced by yeast-cells.*

Observations with Glycerine Extracts of Tissues

Under the impression that the reductase would more readily or perfectly cling to material of comparatively large molecule such as glycerine than to dilute saline solutions, I crushed a fresh blood-free liver (sheep) in glycerine and water (equal parts) and left the crushed material to soak for three days. Three c.c. of the filtered glycerine extract added to ten c.c. of dilute Prussian blue almost entirely bleached the

*According to unpublished information kindly communicated to me by Professor Adrian Brown.

pigment in eighteen hours: the surface layer remained blue. A few cubic centimetres of the reduced material were at once restored by hydrogen peroxide. From this I inferred that the glycerine had extracted both the endo-enzymes, the oxidase and the reductase. This was confirmed by my finding that on boiling the glycerine extract, it completely failed to reduce Prussian blue under the usual conditions, even after a few days' contact.

Methylene blue was reduced to a pale green by the glycerine extract of liver. I noted that the boiled, filtered, glycerine extract used as controls in these experiments had a great tendency to adsorb the respective pigments, though it did not reduce them.

The hepatic glycerine extract in a day or two became acid and smelt sour. Suspecting lactic acid, I neutralised the extract with sodium carbonate, and found that the neutral liquid reduced Prussian blue at 40° C. with the typical white and blue appearance. The previous reduction was thus not due to the presence of acid; and I found that at any rate fermentation lactic acid does not reduce Prussian blue.

Very similar results were obtained by using glycerine extract of crushed blood-free kidney (sheep). This extract reduced Prussian blue to a white, methylene blue to a pale green, indigo blue to a paler blue: this last-mentioned salt was the least affected. The behaviour of the boiled glycerine extract of kidney juice is rather interesting. Neither methylene blue nor indigo blue was reduced by it, but in each case the pigment was adsorbed. When, however, *boiled* glycerine extract was added to Prussian blue the colour was bleached by the third day: it was revived by hydrogen peroxide. Enough reductase had survived the action of the heat to effect a slow reduction but of Prussian blue only—the most easily reduced of the three pigments. To some extent therefore the glycerine shielded the endo-enzyme from destruction by heat.

The superiority of a glycerine over a saline extract was shown in the case of the sheep's liver. This had been cut into pieces and dried at 40° C. six months previously. It was then extracted with ether at least twelve times. [The ethereal extract does not reduce.] The dried, fat-free residue ground up with one per cent. sodium chloride and left in contact with Prussian blue for two days reduced it to a blue-green within six hours, whereas the glycerine and water extract of the dried residue (after two days' contact) made the Prussian blue of a very pale green colour after twenty-four hours' incubation. The boiled glycerine extract had no effect whatever. Some of the fat-free residue itself on being added to

the glycerine extract adsorbed much of the pigment and presented the white-and-blue appearance. The subsequent addition of the original solid matter itself did not seem to increase the velocity of the reduction, so that there was no such relationship as that of ferment and kinase.

Within three months the reducing power of the glycerine extracts had almost disappeared, but they still gave the xanthoproteic reaction. There is evidence that glycerine extracts, especially of kidney, contain more oxidase than saline extracts: boiling destroys the oxidase in all cases. Oxidase was assumed when guaiacum and hydrogen peroxide gave a blue colour with the juice or extract.

In reviewing the experimental work done to demonstrate the existence of a reducing endo-enzyme in animal tissues, one must conclude that up to the present the verdict is 'not proven.' But the evidence for the existence of a reductase is just as good as, and is in some respects better than, that for the existence of certain ferments now recognised, e.g. xanthase, adenase, carbonase, hydrogenase, or the autolytic ferment.

If we follow A. Heffter, protein even in the form of crystallised ovalbumin is autoxidisable by reason of the existence in it of one or more S·H groups, the mobile H of which performs strong reductions. Thus for Heffter neither an oxidase nor a reductase is necessary. Colloids can do all that is attributed to 'respiratory enzymes.' While this may be excellent criticism chemically, it is a protest against the prevailing conception of the distribution of the various vital activities amongst distinct enzymes, some extra- some intra-cellular. Protests of this kind have been made before.* To Heffter, respiratory enzymes are quite superfluous.

Now there is gradually being accumulated a good deal of evidence for the existence of respiratory enzymes. Vernon[31] wrote in 1908 that although 'we are supremely ignorant as to the connection of the oxidases and peroxidases with tissue respiration,' yet we are to hope 'that the connecting-links may be discovered in course of time.' To explain everything capable of being effected by protoplasm in terms of chemical activity alone is not to assist in the elucidation to which Vernon refers, nor to give us any more detailed view of the modus operandi of vital procedures. On Heffter's view the mobile H freed from an S·H grouping

*A. MacKendrick, 'Dissociation in certain vital phenomena,' *Journ. Anat. and Phys.*, Vol. XXXIX, p. 285, 1905.

is all that is needed to effect reductions and convey oxygen thus—

$$2\,(R\cdot SH) + S = H_2S + \begin{matrix} R\cdot S \\ | \\ R\cdot S \end{matrix} \text{ and}$$

$$2\,(R\cdot SH) + O_2 = H_2O_2 + \begin{matrix} R\cdot S \\ | \\ R\cdot S \end{matrix} \text{ and}$$

$$2\,(R\cdot SH) + H_2O_2 = 2\,(H_2O) + \begin{matrix} R\cdot S \\ | \\ R\cdot S \end{matrix}$$

Heffter attributes these powers to cystëine or some similar compound mercaptan, and Rey-Pailhade freely admits that cystëine possesses all the characteristics of ' philothion,' an alleged ferment on which he has written extensively.

While Rey-Pailhade by no means disregards oxidase in his speculations regarding tissue-respiration, oxidase is as unnecessary to Heffter as is a reducing ferment. One would expect blood-serum to have active reducing powers if, as Heffter insists, pure proteins can perform all the reductions attributed to endo-enzymes.

As judged by the same pigments used to test press-juices—Prussian blue and methylene blue—blood-serum has a most trifling power of reduction, probably sufficiently accounted for by the dextrose and other reducing substances in solution. Thus serum (aseptic) dialysed against distilled water does not reduce either pigment in the slightest degree.

Neither serumalbumin alone nor serumglobulin alone has any reducing power on these two pigments. It has been long known that these and other pigments injected into the blood are not reduced in it, though in and by the tissues they are.* This seems against Heffter's view.

A Theory of the Action of Reductase

I venture to identify reductase with the elusive ' oxygenase of protein character which takes up molecular oxygen with formation of peroxides.' According to Moore and Whitley,[21] the evidence for an ' oxygenase ' producing a peroxide is flimsy. But if we assume the endo-enzyme reductase is none other than the so-called oxygenase which becomes the

*I have noticed that normal serum heated for two to five minutes at 100° C. does acquire some reducing power as also the liquid filterable from the heat-coagulum. I have as yet arrived at no satisfactory explanation of this. If heated for several hours, the serum on the other hand has no reducing power.

organic peroxide on assuming its charge of oxygen, then we account for—

(1) The existence of an enzyme which carries oxygen and yet is not itself a 'peroxidase';

(2) The existence of an 'organic peroxide';

(3) A tissue-oxidase or peroxidase simultaneously present along with a reductase.

Vernon holds that the tissues contain a substance which can absorb oxygen from their surroundings to form an organic peroxide. There seems a high probability that this 'substance' and tissue-reductase are identical. Number one in the above classification is a protein or closely allied to such, while all workers seem agreed that number three is not. Provisionally, I suggest that the organic peroxide is the reducing ferment with its burden of oxygen in the act of handing it over to the peroxidase to apply it to the oxidation of the carbon and hydrogen in or in the immediate vicinity of the living bioplasm.

Summary

1. The reductions effected by the press-juice of liver and of kidney are not due to products of putrefaction or autolysis. The reductions are most energetic when the juice is recently pressed: these juices kept aseptic in glass under toluene gradually lose their reducing power: by the end of three months they are inactive.

2. The press-juice is much more active as regards reduction than pieces of the organ itself: in this respect reductase resembles glycogenase,[20] an undoubted endo-enzyme.

3. The reducing power is completely inhibited but not destroyed by temperatures from 0° C. to − 14° C.; its optimum is from about 42° C. to 46° C.; at about 70° C. it begins to be destroyed; it is completely destroyed at 100° C.

4. Reductase is inactivated by treatment with alcohol and by drying, and more so in press-juice than in the intact cells.

5. It is relatively insoluble in that it will not dialyse away from the cell-proteins; it is precipitable along with them by ammonium sulphate.

6. The simultaneous presence of oxidase or a peroxidase must be remembered. It accounts for the re-blueing of the surface of the leuco mixtures of press-juice and pigments. Surface oxidation may be indefinitely postponed by an atmosphere of hydrogen, and almost as perfectly by a layer of olive oil at least one inch thick.

7. There is some evidence that the heat-destruction temperatures are not the same for the two enzymes; for certain glycerine extracts, after being boiled for three to five minutes, produced a leuco condition without any subsequent surface re-blueing. This is in agreement with what Spitzer found as to reducing ferments being still active after boiling, and with the difficulty of Moore and Whitley in destroying certain peroxides by boiling.*

8. Glycerine extracts, both of liver and of kidney, seem to have a greater reducing-power than normal saline extracts, but to undergo a similar deterioration in the same interval of time.

9. Judged by the criterion of solubility, the reducing endo-enzyme has not been isolated. In the opinion of certain biological chemists insoluble endo-enzymes do exist, and that therefore the condition of solubility is too rigorous a test. Reductase would belong to this class. Certainly 'extracts' (if they are not solutions) of some active constituent of liver and kidney possess a power of reduction which is diminished with lapse of time and is injuriously affected by certain reagents and agencies in a manner wholly comparable with that of substances long recognised as enzymes.

10. The deteriorated substance is still a protein.

I am grateful to Professor Carlier and Professor Adrian Brown, of the University of Birmingham, for their interest in the various phases of the problems dealt with, and must express my indebtedness to Mr. Lovatt Evans, of the Physiological Department, for both advice and ever willingly given assistance.

Loc. cit., p. 139.

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ON THE PRESENCE OF INSOLUBLE SALTS OF CALCIUM (OXALATE AND PHOSPHATE) IN RENAL CALCULI IN LARGE AMOUNT IN A PREPONDERATING NUMBER OF CASES, AND THE BEARING OF THIS FINDING UPON CALCIUM METABOLISM IN GOUT AND ALLIED CONDITIONS

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In a previous paper from this laboratory by J. S. Rowlands[1] it was pointed out from qualitative experiments on a considerable number of renal calculi, that the usual view that a considerable percentage of such stones are very rich in urates of an insoluble character is erroneous, for in a majority of the cases uric acid could not be demonstrated at all by the usual qualitative tests, and in only two or three stones of the series was any appreciable amount of uric acid present. The preponderating number of the stones were, on the other hand, found to contain calcium oxalate and phosphate in very considerable amount.

The current view that a large percentage of kidney stones contain urates in high amount is important from two aspects. In the first place the supposed urate content is placed in correlation with the clinical observation that renal calculi occur most frequently in gouty subjects, and the gouty diathesis is set down as the cause for the formation of these concretions supposed to be very rich in insoluble urates.

Secondly, such supposed urate concretions are used as a support for the very popular view that the faulty nucleic acid metabolism and associated production and accumulation of urates and allied bodies is the chief factor in the causation and symptomatology of gout.

1. This Journal, Vol. III, p. 346, 1908.

Now, if it is a fact which can be experimentally demonstrated that renal calculi nearly always consist not of insoluble urates, but mainly of calcium oxalate and calcium phosphate in varying ratios, a new and important standpoint in relationship to gout is established, which may be of considerable importance, both in understanding, and in treating that condition.

Such a result would indicate that the calcium metabolism in gout may be more at fault than that of the purins and nucleo-proteins, and would give a new aspect to both dieting and treatment.

The calcium oxalate, it is true, would indicate a diminished oxidizing power of the organism, just as does uric acid and the allied purin bases; and the disturbed nuclear metabolism would yield the accumulation of calcium phosphate from the rich phosphorus content of the nucleic acids of the nuclei. So that, in one way, the oxalate and phosphate might be produced in the same pathological derangement which would lead to uric acid accumulation. Also, the calcium salt of uric acid is one of the least soluble of the urates, as shown by Little, so that calcium urate, phosphate and oxalate might readily occur in association.

Still, the presence of calcium, as practically the only base present in such calculi, is an experimental point of considerable importance which seems to us to be little known, and still less appreciated.

It may well be that calcium is as much or more to be avoided in treating gout as are the much dreaded purins and uric acid producers, and it is interesting from this point of view to note, in passing, that nearly all wines contain inorganic calcium salts in considerable quantity, due to treatment of the wines to free them from tartaric acid and other organic acids. Milk in various forms and cheese with a high calcium content are rarely prohibited to the gouty, and yet may, from the evidence we shall give, be more dangerous than other protein food; and our experience is that gouty subjects cut off from other proteins have usually acquired the habit of consuming cheese in increased quantities.

As regards treatment for prevention of gouty concretions, if these, like the renal calculi, are rich in calcium oxalate and phosphate, then the alkaline treatment is the exact opposite of what is required, for these bodies, unlike the urates (including calcium urate), are insoluble in alkali and soluble in acid.

But, whatever may be the pathological and clinical bearings of the problem, it is obviously of considerable experimental importance

to determine in a considerable number of cases as closely as possible the quantitive chemical composition of renal calculi.

For this purpose we have quantitatively analysed, and describe in this paper, a series of twenty-four renal calculi, taken indiscriminately as they came, twenty-one of the series being from the practice of one surgeon (W. T. T.).

The water supply of the district inhabited by most of the patients, viz., Liverpool district and North Wales, may be taken as a rule as being very soft surface water containing very little calcium salts, so that there is no reason to suppose that the high percentage of calcium oxalate and phosphate found in the great majority had any origin in the water supply.

There is no other cause, which we can trace, why this series of stones should differ in chemical composition from a similar series taken from any other district, and we are at a loss to find a basis for the usual text-book description of the so-called urate stone.

Only two of the stones, both found in the bladder, consisted almost wholly of urates, and it is interesting to the radiographer to note that these stones, both inside and outside the body, were transparent to the X-rays, while all the others, with one exception on one occasion, were opaque and diagnosable by X-ray methods.

Gall stones are poor in or lacking entirely in calcium salts, and, as we shall show, renal calculi consist nearly always almost wholly of calcium salts (oxalate and phosphate), and herein lies the reason that renal calculi show up with X-ray illumination, while biliary calculi are invisible or almost so.

Taking our series as a whole in regard to urate content, uric acid is so low as to give no positive or a very uncertain result in many cases; and to quantitative methods *even if all the nitrogen present be taken to be urate nitrogen*, the calculated uric acid lies away down at 2 or 3 per cent., and rarely over 10 per cent. Estimation of the uric acid by permanganate titration in the portion insoluble in moderately strong hydrochloric acid, leads to still lower results, and in several cases it is obvious that the small amount of organic nitrogen present is there as protein and other non-urate forms.

In describing the analyses of each stone in our protocolls, we give a very brief summary of the history of the case, and a concise description of the macroscopic appearance and physical properties of the stone.

After a cast of the stone had been prepared in each case, the whole

stone was powdered up and used for the analyses; so that a good average sample of each calculus was obtained for the quantitative work.

Methods of Analyses

Moisture. After the physical characteristics of the stone had been noted, it was weighed carefully and then crushed and ground to a fine powder in a mortar, noting as far as possible the degree of hardness. The powder was weighed on a watch-glass, and then dried for about two hours at 100° C. and again weighed, the loss in weight being taken as moisture. The analyses proper were then carried out upon the dried material, the first process being a thorough qualitative analysis.

Qualitative testing. In each case ammonia, xanthine, cystine, magnesium and sulphates were tested for carefully, and invariably found to be absent.

Uric acid and total nitrogen. Uric acid was tried for by the murexide test, with negative results in some cases and feeble positive results in some others. Uric acid was determined by titration in sulphuric acid solution with N/20 potassium permanganate, in the residue obtained after treatment of a weighed amount of calculus with dilute hydrochloric acid (1 in 4 of strong hydrochloric acid solution). Also, in certain cases the uric acid was estimated by Hopkin's method, but as the results almost coincided with those obtained by direct titration after acid treatment, this simpler method was more often employed. As a control, the total nitrogen in another weighed sample of the stone was determined by Kjeldahl's method; this result in some cases lay near to the uric acid nitrogen value and in others was considerably higher, showing that nitrogen was present in other forms than urates. In stating the results below, in each case total nitrogen is calculated out as if it were present as uric acid. This is done not on the idea that all the total nitrogen is uric acid, which would be a very erroneous basis for some of the calculi, but rather to show that even on this simple basis of all the nitrogen being urate nitrogen, the percentage is still much too low to countenance the conception of a urate stone.

Calcium oxalate and calcium phosphate. The determination of oxalates was carried out by precipitation as calcium oxalate, filtering by the Bunsen pump through a Gooch filter and dissolving in sulphuric acid and titrating with N/20 potassium permanganate. The amount of phosphate was estimated as P_2O_5 by destroying organic matter by

Neumann's method of oxidation with strong sulphuric and nitric acids, then precipitating by ammonium molybdate, filtering, breaking up the phospho-molybdate by alkali, and titrating back with N/10 alkali, as described below.

The order of events in carrying out the various analyses was as follows:—

A quantity, varying according to the amount of stone available, was taken and accurately weighed. This was then placed in a porcelain dish, treated with hydrochloric acid (1 in 4 of strong solution) and heated on a steam bath until as much as possible had been dissolved. It was next filtered through a weighed Gooch filter, the residue well washed, and then dried at 100° C. for from one to two hours until the weight was constant, and this was noted. The residue in the Gooch, asbestos and all, was next treated with strong sulphuric acid. The acid was then diluted and titrated with N/20 potassium permanganate solution, 1 c.c. of which equals 0·0075 gramme of uric acid, and the amount thus estimated. A control had always to be performed with the sulphuric acid and water alone in order to get an accurate result, the amount of permanganate taken by the control being deducted from the result of the former titration.

This method of estimating uric acid was found to be fairly accurate when tested with a pure uric acid stone. Hopkin's method was adopted, as above stated, in three or four of the stones, but it is a somewhat lengthy process, and for the small amounts of uric acid, already in insoluble form, which were dealt with, it was not found to be any more accurate than this direct method.

The filtrate obtained after digesting the stone with hydrochloric acid and the washings of the residue were next mixed, and ammonium chloride and ammonia added in excess, the whole boiled, and whilst boiling ammonium oxalate was added. This was the procedure in the majority of the stones; in a few, however, the precipitate obtained after adding ammonium chloride and ammonia was filtered, thoroughly washed until free from chlorides, the washings being tested with silver nitrate, and then dried and weighed. The filtrate obtained after the addition of ammonia was then boiled and ammonium oxalate added as before. The precipitate thus obtained was allowed to settle, the liquid filtered off, and the precipitate washed by decantation until free from chlorides, which would otherwise interfere with the permanganate titration. The precipitate then, in a few of the stones, was dried and

weighed, but in the majority it was washed from the filter paper into a beaker by means of hot water first, then fairly strong sulphuric acid, and finally all the acid was washed into the beaker by means of hot water. The whole was then thoroughly mixed, cooled, and made up to 500 c.c. or 1,000 c.c., and 50 c.c. pipetted and titrated with N/20 potassium permanganate solution, 1 c.c. of which was equivalent to 0·0019 gramme of calcium or 0·00608 gramme of calcium oxalate.

Another portion of the stone was now weighed out, and the total nitrogen estimated by the Kjeldahl process.

Phosphates were estimated as phosphorus pentoxide by the following method:—A portion of the powdered stone having been weighed was put into a Kjeldahl's flask and 20 c.c. of a mixture of equal parts of strong sulphuric and strong nitric acids added, and the whole boiled until quite clear, as recommended by Neumann; if it was not clear at the end of about two hours a further 10 c.c. of the acid mixture was added and the boiling proceeded with. When quite clear it was made up to 150 c.c with distilled water and again boiled. Whilst boiling 50 c.c. of 50 per cent. ammonium nitrate and 30 c.c. of 10 per cent. ammonium molybdate were added. A yellow precipitate appeared, in the presence of phosphates, and the whole was allowed to cool. The liquid was filtered off, and the precipitate washed by decantation with cold distilled water until quite free from acid. If much of the precipitate is allowed to get into the filter the filtering becomes a very tedious process. The whole precipitate, together with the filter paper, was next transferred back into the flask, and the whole dissolved in standard decinormal sodium hydrate solution. When the precipitate was entirely dissolved, the liquid was boiled for about fifteen minutes to get rid of the ammonia, cooled and titrated with decinormal sulphuric acid, phenolphthalein being used as indicator. We in this way found the amount of sodium hydrate required to dissolve the phosphomolybdate precipitate, and know that 1 c.c. of the N/10 sodium hydrate solution is equivalent to 0·2325 milligramme of phosphorus pentoxide. The amount of calcium phosphate was then obtained from this. As calcium was practically the only base found present by exclusive qualitative analysis, the estimations and calculations were greatly simplified.

The details were necessarily modified in analysing some of the stones, but these modifications will be indicated in the account which follows, of the individual stones. A brief history of the length of time

of the symptoms, together with the district from which the patient came, is also given.

Details of Analyses of the Twenty-four Calculi

Calculus No. I. [R. W.] A male, age 17, residing in Liverpool. He had had symptoms of stone for nine years. Neither blood nor pus were ever found in the urine. The stone was removed from the right kidney. It weighed 6·202 grammes, was smooth, dark brown in colour, extremely hard, the hardest in fact of the whole series, and great difficulty was experienced in dissolving it. It contained 2 per cent. moisture, and qualitatively consisted of calcium oxalate and phosphate and uric acid. In order to dissolve it, it was left in hydrochloric acid (1 in 4) for four days, but only 20 per cent. dissolved; strong hydrochloric acid was next used and 30 per cent. more dissolved; a total of 54 per cent. of the dried stone was thus dissolved in the acid, and of this 50·14 per cent. was found to be calcium oxalate.

The total nitrogen determined by Kjeldahl yielded from 0·951 gramme of stone 0·038 gramme. If all this be taken as uric acid, it calculates out at 11·9 per cent. of the dried stone.

By the ordinary method described above 9·9 per cent. of uric acid was obtained.

Another portion of the stone, 0·339 gramme, yielded 0·00263 gramme of phosphorus pentoxide, or 1·7 per cent. of calcium phosphate.

From a percentage point of view these results are the most unsatisfactory of the whole series, and being the first they were rather discouraging; but even later, when several calculi had been examined, on coming back to this one no better results were obtained. By incinerating a portion and estimating the calcium, 0·579 gramme of the stone yielded 0·1216 gramme of calcium or 67·2 per cent. oxalate, and by boiling 0·878 gramme of the stone with strong hydrochloric acid it yielded a higher figure still, namely, 0·19 gramme of calcium or 69·12 per cent. of oxalate.

No difficulty was experienced in dissolving any of the subsequent calculi, and the oxalate in this had undoubtedly been deposited in a very insoluble form.

Calculus No. II. [M. M.] A female, age 44, residing in St. Helens. She had had symptoms of calculi for eleven years; blood was noticed in the urine twice, never any pus. The stone was removed from

the left kidney. It weighed 1·691 grammes, was rough, light brown in colour, soft, very friable, and contained 17·9 per cent. of moisture. Qualitatively it consisted of calcium oxalate and phosphate, and only a trace of uric acid.

For the estimation of calcium oxalate 0·23 gramme of the stone was taken, yielding 0·0285 gramme of calcium or 87·3 per cent. of oxalate, and 4·27 per cent. uric acid by the direct method. By a Kjeldahl 0·192 gramme yielded 0·005 gramme of nitrogen, or as uric acid 7·8 per cent. The phosphorus pentoxide obtained from 0·471 gramme of the stone was 0·0126 gramme or 5·8 per cent.

Calculus No. III. [E. T.] A male, age 43, residing in Cheshire. He had suffered from symptoms of stone for two years. Haematurea was noticed once; never any pus. The stone was removed from the left kidney. It weighed 3·562 grammes, was rough, brownish white in colour, and very hard. It consisted chiefly of calcium oxalate, with a small percentage of uric acid and phosphate, and contained 3·5 per cent. moisture. A Kjeldahl yielded 0·0056 gramme of nitrogen from 0·208 gramme of the dried stone, or if all was present as uric acid 8 per cent.

A calcium oxalate estimation yielded 0·0589 gramme of calcium from 0·217 gramme of stone or 87·6 per cent. of calcium oxalate.

The phosphorus pentoxide yield from 0·383 gramme was 0·01298 gramme or 7·4 per cent. calcium phosphate.

By the direct method 6·9 per cent. uric acid was found.

A difficulty presented itself at this stage for the first time. In the previous two stones allowance had been made in the first figures for the calcium which was there as phosphate. This having been found, it was deducted from the amount obtained for the oxalates. It was thought that the calcium phosphate on the addition of ammonium oxalate would be changed into calcium oxalate and precipitated as such, but in this case it was noticed that although only about 7 per cent. of the stone remained undissolved, only 86·7 per cent. of calcium oxalate was obtained as a precipitate. This meant that although it was dissolved by the hydrochloric acid, about 7 per cent. of the stone was unaccounted for. A portion of the stone was incinerated, but this gave a similar result. This difficulty remained unsolved until the eighth stone of the series was being estimated, when, although no other base could be discovered, the calcium was only 9·84 per cent. by this method, which would only give a 30 per cent. yield of calcium oxalate if all the calcium was in the stone as oxalate, and there was still a large amount of phosphates to be

provided with a base. It then occurred to us that the phosphates were precipitated by the ammonium hydrate as phosphates, and consequently, when the ammonium oxalate was added later, it had no effect on them, and this was found to be the case, and the difficulty was solved. The permanganate only estimated that amount of calcium in the stone which was there as oxalate, and consequently the figures obtained by this method had to be taken as oxalate alone, the amount which combined with the phosphates being calculated from the figures obtained from the estimation of phosphorus pentoxide.

Calculus No. IV. [M. L.] A female, age 26, residing in Wigan. There was a history of symptoms of calculus for twelve months. The stone was removed from the right kidney. It weighed 1·063 grammes, was rough on the surface, friable, light yellow in colour, and contained 6·8 per cent. moisture.

Qualitatively it consisted almost entirely of calcium oxalate, with a trace of calcium carbonate and a very small amount of uric acid.

A Kjeldahl yielded 0·0042 gramme of nitrogen from 0·156 gramme of stone, or 7·68 per cent. uric acid if all the nitrogen was there as such.

By the direct method there was a yield of 1·7 per cent. uric acid.

The calcium obtained from 0·118 gramme was 0·038, which gives a percentage of 99 if all was as oxalate, but there was a small portion carbonate, which accounts for the high figure.

Calculus No. V. [W. L.] This was a male, age 27, residing at Litherland. The symptoms of calculus had commenced twelve months before. Neither blood nor pus were at any time found in the urine. The stone was removed from the right kidney. It weighed 0·673 gramme, was rough on the surface, friable, brown in colour, and contained 2·72 per cent. moisture.

It was practically a pure calcium oxalate stone, no uric acid being demonstrable either by qualitative or quantitative tests.

By a Kjeldahl 0·084 gramme of stone yielded 0·0014 gramme of nitrogen, which, being calculated as uric acid, gives 3·9 per cent.

No uric acid was found by the direct method.

The yield of calcium from 0·076 gramme of stone was 0·02375 gramme, or, if calculated as oxalate, 99·77 per cent., but here again a little carbonate was present.

Calculus No. VI. [W. A.] A male, age 23, residing in Wrexham. He gave a history of symptoms lasting two years. Blood was found in the urine once; never any pus. The calculus was removed from the lower

end of the ureter. It weighed 14·222 grammes, was smooth on the surface, dark brown in colour, extremely hard, and contained 2·43 per cent. moisture. Qualitatively it consisted chiefly of calcium oxalate, with a small quantity of phosphate and only a very faint trace of uric acid.

In the estimation of calcium 0·4 gramme of stone yielded 0·1064 gramme, or 85·12 per cent. of oxalate, and by the direct method 1·5 per cent. uric acid.

By a Kjeldahl 0·653 gramme yielded 0·00616 gramme of nitrogen, which gives 2·82 per cent. uric acid if all the nitrogen is present as such.

There was 0·0256 gramme of phosphorus pentoxide from 0·383 gramme of stone, or 14·5 per cent. calcium phosphate.

Calculus No. VII. [J. H. E.] A male, age 55, residing at Hoylake. This patient was X-rayed in London on account of indefinite pain in the left loin, and although no calculus was shown on the plates, he was advised to have the kidney explored. Again in Liverpool he was X-rayed, and, in spite of good negatives being obtained, no calculi were shown by them. On sounding the bladder, however, stones were discovered, and this one was one of eight removed. It weighed 0·443 gramme, was fairly smooth on the surface, soft and friable, pale pink in colour, and contained 6·5 per cent. moisture.

Qualitatively uric acid alone was found, and, being estimated quantitatively by Hopkin's method, 0·121 gramme yielded 97·5 per cent. pure uric acid.

This, therefore, is purely a uric acid stone; it was found in the bladder and was transparent to X-rays.

Calculus No. VIII. [M. C.] A female, age 22, residing in Liverpool. This patient was X-rayed on account of symptoms pointing to stone in the right kidney, but although good negatives were obtained, no shadow was shown on them. Sixteen months later, she came back with similar symptoms, and an X-ray plate now taken showed a calculus at the lower end of the right ureter. It weighed 4·405 grammes, was friable, a light yellow in colour, and contained 13·2 per cent. of moisture.

Qualitatively it consisted of calcium phosphate and oxalate, and a small amount of uric acid.

The phosphorus pentoxide yielded by 0 39 gramme of stone was 0·1081 gramme or 60·9 per cent. of calcium phosphate. From 0·386 gramme of the stone 0·038 gramme of calcium was obtained or 31·48 per cent. calcium oxalate, and by the direct method 6·73 per cent. of uric acid.

A Kjeldahl yielded 0·02914 gramme of nitrogen from 0·416 gramme of the stone, or, calculating it all as uric acid, 21·01 per cent. This was a very high reading, and can only be accounted for by the supposition that the stone when ground up was not thoroughly mixed, and that the nucleus or other portion which contained a large percentage of uric acid was taken for this Kjeldahl estimation. This reading should in all probability be rejected, and the lower figure given by the portion taken for the oxalate estimation be accepted.

Calculus No. IX. [H. F.] A male, age 21, resident in Liverpool. He stated that he had had the symptoms which he then complained of ever since he could remember. The calculus was the larger of two removed from the kidney. It weighed 1·712 grammes, was rough, very dark brown, and very hard, and contained 1·5 per cent. moisture. It was composed of calcium oxalate, uric acid, and a trace of phosphate.

The amount of calcium obtained from 0·374 gramme of the stone was 0·0931 gramme or 79·6 per cent. By the direct method 10·6 per cent. of uric acid was obtained. A Kjeldahl gave 0·01792 gramme of nitrogen from 0·384 gramme, or, reckoned as uric acid, 14 per cent.

The amount of phosphate was very small, 0·403 gramme of the stone yielding only 0·0035 gramme of phosphorus pentoxide or 1·9 per cent. of calcium phosphate.

Calculus No. X. [T. L.] A male, age 55, residing in Cardiganshire. His symptoms had lasted two years. He had noticed blood in his urine once; pus was not found on any occasion. The calculus was removed from the right kidney. It weighed 1·143 grammes, was rough, crystalline, hard, and contained 1·9 per cent. moisture.

Qualitatively its chief constituent was calcium oxalate together with a small quantity of phosphate and uric acid.

There was 0·077 gramme of calcium in 0·271 gramme of the stone, or 90 per cent. calcium oxalate, and 4 per cent. uric acid by the direct method. In 0·239 gramme, 0·01 gramme of phosphorus pentoxide was found, or 8·7 per cent. calcium phosphate, and by a Kjeldahl 0·247 gramme yielded 0·00308 gramme of nitrogen, or, calculated as uric acid, 3·6 per cent.

Calculus No. XI. [E. T.] A female patient, age 39, residing in Liverpool. She had suffered from pain, haematuria and pyuria for ten years. The calculus was found in the right ureter. It weighed 0·483 gramme, was rough, dark brown in colour, hard, and contained 1·9 per cent. moisture.

There was insufficient of the stone to do a qualitative analysis, and the following modifications were adopted in the quantitative:—

For a Kjeldahl 0·205 gramme was taken, which yielded 0·0049 gramme of nitrogen, or, calculated as uric acid, 6·8 per cent. Then 0·197 gramme was heated with hydrochloric acid on a water bath and filtered, the residue being dried and weighed, and by the direct method yielded 2·5 per cent. uric acid. The filtrate was treated as before for calcium oxalate, but instead of dissolving the precipitate, which was obtained on the addition of ammonium oxalate, with strong sulphuric acid, it was dried and weighed, and then this precipitate was tested for phosphates. If any phosphates had been found, which they were not in this case, they would have been estimated in this precipitate, as was done in some of the later calculi, and the amount of oxalate obtained by difference.

From this 0·197 gramme, 0·186 gramme of calcium oxalate was obtained, or 94·4 per cent.

Calculus No. XII. [C. H.] A male, age 50, residing at Oldham. Calculi had been twice removed from the bladder, but a shadow had never been obtained on an X-ray plate. This one was no exception; it also did not give a shadow on the negative.

It was removed from the bladder, weighed 6·224 grammes, was smooth on the surface, reddish brown in colour, and contained 9 per cent. moisture.

It was found on analysis to be a pure uric acid stone. A Kjeldahl yielded 0·0522 gramme of nitrogen from 0·158 gramme of stone, or 98·5 per cent. uric acid.

Calculus No. XIII. [F. R.] A male, age 40, a resident of Liverpool. Blood and pus had been found in the urine, and oxalates in great quantities. The calculus was removed from the right kidney. It weighed 11·365 grammes, was rough, having white crystals in places, the main portion of the stone being dark brown. It was hard, and contained 2·5 per cent. of moisture.

Qualitatively it consisted entirely of calcium oxalate and phosphate, no murexide reaction being obtained.

An estimation of calcium in 0·925 gramme of the stone gave 0·2489 gramme, or 86·1 per cent. calcium oxalate. By the direct method 1·8 per cent. uric acid was obtained, and by a Kjeldahl 0·0072 gramme of nitrogen was found in 0·716 gramme of stone, which, being calculated as uric acid, gives 3 per cent. The phosphorus pentoxide was estimated in

0·768 gramme, which yielded 0·02346 gramme, or 6·6 per cent. calcium phosphate.

Calculus No. XIV. [A. S.] A male, age 34, residing in Liverpool. His symptoms had lasted for four years. Both haematuria and pyuria were present. It was the larger of two stones removed from the left kidney.

It weighed 1·028 grammes, was very rough, crystalline on the surface, light coloured, except in one place where it was dark brown, and contained 2·1 per cent. moisture.

Qualitatively it consisted of calcium oxalate and phosphate, and again no murexide reaction was obtained.

The calcium oxalate yield was 84·7 per cent., 0·323 gramme of stone yielding 0·0855 gramme of calcium. By the direct method 3·7 per cent. uric acid was obtained, and from a Kjeldahl 4 per cent. uric acid, 0·0045 gramme of nitrogen being found in 0·334 gramme of stone. Calcium phosphate was estimated at 13 per cent., 0·314 gramme yielding 0·01879 gramme phosphorus pentoxide.

Calculus No. XV. [L. R.] A male, age 22, residing in Pwllheli. There was a history of symptoms of calculus for two years. Haematuria and pyuria were both present. X-rays showed the presence of calculi in both kidneys. This calculus was removed from the right kidney. It weighed 6·533 grammes, was rugged and soft externally, but very hard in the centre; it was light brown in colour, and contained 12·8 per cent. moisture.

In 0·453 gramme, 0·0732 gramme of calcium was found, or 51·7 per cent. calcium oxalate, and in 0·395 gramme, 0·073 gramme of phosphorus pentoxide, or 40·4 per cent. calcium phosphate. A Kjeldahl yielded 0·0063 gramme of nitrogen from 0·449 gramme of stone, or, calculated as uric acid, 4·2 per cent., and the direct method yielded a percentage of 2·4.

Calculus No. XVI. [J. G.] A male, age 22, residing at Ruthin. He had had pain for two years. Haematuria was present, but not pyuria. The calculus was removed from the left kidney. It weighed 0·744 gramme, was rough, dark brown in colour, very hard, and contained 1·7 per cent. moisture.

Exactly the same method was employed with this as with No. XI.

A Kjeldahl yielded 0·0021 gramme of nitrogen from 0·287 gramme of stone, being equivalent to 2·19 per cent. uric acid. By the direct method 5·8 per cent. uric acid was obtained. The calcium oxalate yield was 0·335 gramme from 0·357 gramme of stone, or 93·8 per cent. No phosphate was found.

Calculus No. XVII. [M. W.] A male, age 33, residing in North Wales. He had suffered from pain in the left side for ten years. Four years ago he had pain in the right side for a short time, but this left him after the passage of a small calculus. Haematuria and pyuria were present. The calculus was removed from the left ureter. A similar method to the one used in the case of the previous stone was employed, except that the portion which remained undissolved by the hydrochloric acid had the nitrogen in it estimated by the Kjeldahl process, and no other Kjeldahl was done. The calculus weighed 0·366 gramme, was irregular, dark brown in colour, very hard, and contained 3·9 per cent. moisture.

In 0·341 gramme there were found 0·0044 gramme of nitrogen, or, calculated as uric acid, 3·8 per cent., 0·0746 gramme of phosphorus pentoxide or 47·8 per cent. calcium phosphate, and 0·141 gramme of calcium oxalate or 41·3 per cent. This latter result was obtained by difference, the whole precipitate, after adding ammonium oxalate, being 0·304 gramme.

Calculus No. XVIII. [G. W.] This was one of three ureteral calculi. It weighed 0·968 gramme, was soft and crumbling, light coloured, and contained 15·7 per cent. moisture. No qualitative analysis was done in this or in any of the succeeding calculi, on account of the small quantity available.

A Kjeldahl yielded 0·0054 gramme of nitrogen from 0·231 gramme of stone, or 6·9 per cent. uric acid. By the direct method 9·6 per cent. uric acid was obtained.

In 0·325 gramme of the stone 0·0171 gramme of calcium was found, or 16·8 per cent. of calcium oxalate; and in 0·261 gramme of the stone 0·0829 gramme of phosphorus pentoxide or 69·3 per cent. calcium phosphate was obtained.

Calculus No. XIX. [E. E.] A female, age 21, residing in Liverpool. She had had symptoms of calculi for eight years. The calculus was removed from the right kidney. It weighed 0·797 gramme, was dark brown in colour, rugged on the surface and hard, and contained 2·6 per cent. moisture.

A Kjeldahl yielded 0·0126 gramme of nitrogen from 0·239 gramme of stone, or calculated as uric acid 15·8 per cent. The percentage of uric acid by the direct method was 11·9.

In 0·21 gramme, 0·0532 gramme of calcium was found, giving 81·04 per cent. calcium oxalate, and in 0·315 gramme of stone 0·006 gramme of phosphorus pentoxide or 4·1 per cent. calcium phosphate was found.

Calculus No. XX. [J. D.] A male, age 31, residing in Liverpool. Twelve months duration of symptoms. There was haematuria, but no pyuria. The calculus was removed from the right ureter. It weighed 0·138 gramme, was soft, light in colour, and contained 3·9 per cent. moisture.

The method employed in this and in the succeeding stones was similar to the one used in No. XVII.

From 0·123 gramme of stone there was yielded 0·00126 gramme of nitrogen, or 3·09 per cent. uric acid, and 0·117 gramme of a mixture of calcium phosphate and calcium oxalate. This latter mixture yielded 0·03583 gramme of phosphorus pentoxide or 63·5 per cent. calcium phosphate, and therefore by difference 0·0388 gramme of calcium oxolate, or 31·6 per cent.

Calculus No. XXI. [H. T.] A male, age 24, residing in St. Helens. There was haematuria, but no pyuria. The calculus was removed from the right kidney. It weighed 0·134 gramme, was rough, dark brown in colour, hard, and contained 9·375 per cent. moisture.

From 0·12 gramme of stone there was obtained 0·00154 gramme of nitrogen, or 3·83 per cent. uric acid, and 0·114 gramme of calcium oxalate, or 95 per cent. There was no phosphate present.

Calculus No. XXII. [M. H.] A female, age 27, residing in Liverpool. The symptoms had lasted twelve months. There was no haematuria or pyuria. The calculus was removed from the right ureter. It weighed 0·134 gramme, was soft, and contained 11·6 per cent. moisture.

From 0·114 gramme of stone there was obtained 0·0042 gramme of nitrogen, or 11·05 per cent. uric acid, and 0·096 gramme of a mixture of calcium oxalate and calcium phosphate, which gave 0·02703 gramme phosphorus pentoxide, or 51·7 per cent. calcium phosphate, and thus leaving 32·5 per cent. calcium oxalate.

Calculus No. XXIII. [A. C.] A male, age 39, living at Crosby. He had previously been X-rayed on account of symptoms of calculi, but none were found. Thirteen months later he was again radiographed, and a definite shadow was shewn on the right ureter. It weighed 0·158 gramme, was rough on the surface, dark brown in colour, hard, and contained 2·7 per cent. moisture.

A Kjeldahl from the residue was spoiled, but taking the whole residue as uric acid it was 0·007 gramme in 0·139 gramme of stone, or 5·03 per cent.

The oxalate and phosphate precipitate weighed 0·131 gramme, and from this 0·00245 gramme of phosphorus pentoxide was obtained, or 3·81 per cent. calcium phosphorus and 90·39 per cent. calcium oxalate.

Calculus No. XXIV. This was one of a number of calculi removed from the substance of the prostate. It was of a light brown colour and fairly hard. It weighed after drying 0·156 gramme. It consisted of 0·088 gramme of calcium oxalate, or 56·4 per cent., and 0·062 gramme of calcium carbonate. There was no uric acid present.

TABLE OF PERCENTAGE COMPOSITION OF THE CALCULI

No.	Origin	Moisture	Calcium oxalate	Calcium phosphate	Uric acid. Direct method	Total nitrogen. Kjeldahl	Total nitrogen calculated to uric acid
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1	R. Kidney	2	69·1	1·7	9·9	3·9	11·9
2	L. „	17·9	87·3	5·8	4·2	2·6	7·8
3	L. „	3·5	86·7	7·4	6·9	2·6	8·0
4	R. „	6·8	99	none	1·7	2·3	7·0
5	R. „	2·7	99·7	none	none	1·3	3·9
6	R. Ur. ter	2·4	85·1	14·5	1·5	0·9	2·8
7	Bladder	6·5	none	none	97·5	—	—
8	R. Kidney	13·2	31·5	60·9	6·7	7·0	21·0
9	R. Kidney	1·5	79·6	1·9	10·6	4·6	14
10	R. „	1·9	93·0	8·7	4·0	1·2	3·6
11	R. Ureter	1·9	94·4	none	2·5	2·2	6·8
12	Bladder	9·0	none	none	—	32·8	98·5
13	R. Kidney	2·5	86·1	6·6	1·8	1·0	3·0
14	L. „	2·1	84·7	13·0	3·7	1·3	4·0
15	L. „	12·8	51·7	40·4	2·4	1·4	4·2
16	L. „	1·7	93·8	none	5·8	0·7	2·2
17	L. Ureter	3·9	41·3	47·8	—	1·2	3·8
18	L. „	15·7	16·8	69·3	9·6	2·3	6·9
19	R. Kidney	2·6	81·0	4·1	11·9	5·2	15·8
20	Ureter	3·9	31·6	63·5	—	1·0	3·1
21	R. Kidney	9·3	95·0	none	—	1·2	3·8
22	R. Ureter	11·6	32·5	51·7	—	3·6	11·0
23	R. Ureter	2·7	90·4	3·8	5·0	—	—
24	Prostate	—	95·1	none	none	—	—

All the percentages, except that of moisture, are calculated in terms of the dried stone.

As pointed out in the text, all the total nitrogen is not present as uric acid; it is shewn in the final column *calculated* as uric acid to furnish an *a fortiori* proof that in nearly all these calculi the percentage of uric acid is a low one.

Analysis of the Tabular Percentages and Discussion of the Bearing of the Results

There are two calculi of the series absolutely different to all the others in chemical composition, viz., Nos. VII and XII, and it is remarkable that these two alone were from the bladder, all the others coming from the kidney, ureter or prostatic pouch. The two bladder calculi were almost pure uric acid, containing no trace of calcium, either as oxalate or as phosphate. All the other calculi contained calcium oxalate in large amount, only one less than 30 per cent., and more than two-thirds of them over 70 per cent. of this insoluble calcium salt. The next most abundant constituent was tri-calcic phosphate, also a very insoluble calcium salt; this constituent reached over 50 per cent. in four out of the twenty-two calculi, and was present in quite appreciable amount in six others.

The most remarkable fact, and perhaps most important, is the strikingly low uric acid percentage in all save the two bladder stones; the figures only reach the neighbourhood of 10 per cent. as a maximum in four out of the twenty-two calculi, when the direct titration method is taken for survey, and even the total nitrogen (Kjeldahl) figures show only slightly higher percentages, with one exception (No. 8), where a good deal of the nitrogen was obviously present in other than urate forms.

The contrast in the two bladder stones is so obvious as to raise doubts in our minds as to whether these two calculi ever were formed in the kidney, and it would be most interesting if these turned out to be concretions of vesicular origin.

It is also interesting to note that these two purely uric acid calculi were transparent to X-rays, and we find that the ordinary kidney and urethral calculi are opaque, because, like bone, they contain these large percentages of insoluble calcium salts (oxalate and phosphate).

The presence of calcium oxalate as an abundant and frequent constituent of renal calculi, in fact, as the chief constituent of such calculi, opens up a field of most interesting thought as to the metabolism of renal calculus formation. In the first place, the incompletely oxidised oxalate, just like uric acid, points unmistakably to embarrassed oxidation or increased reducing conditions attending their formation.

It might be supposed also that if under pathological conditions there is a tendency to production in the system of oxalic acid, then calcium oxalate being the least soluble oxalate, there would be a tendency to

formation of this insoluble salt, followed by its precipitation as a calculus around any available nidus of already precipitated material. Once started in such a system, slightly supersaturated with calcium oxalate in solution, the calculus goes on growing and increasing in size.

However, the fact that calcium oxalate is not the only calcium salt of these renal concretions, but that tri-calcic phosphate, also an insoluble calcium salt, is present and in considerable amount in a fair percentage of the calculi, leads to the view that the same faulty metabolism which leads to excess of oxalic acid also leads to excess of phosphoric acid. This view would be consonant with the importance assigned in such a condition to the uric acid and purin bases, for any exaggerated nuclear metabolism would probably simultaneously yield from the nucleic acids purin derivatives and phosphoric acid, since these bodies are chemically associated in the nucleins.

An alternative view would lay greater stress on the common kation, calcium, of both the oxalic acid and phosphoric acid, and see in the sequence of events a disordered calcium metabolism in which calcium ion in excess was set free and passed into union with those anions in the plasma which would form the most insoluble salts, namely, oxalic, phosphoric, and uric acid anions. This supposition would also explain the association of these three particular acids with calcium in our series of renal calculi.

There is no doubt possible, from abundant collateral evidence, that certain pathological processes associated with insufficient oxidation lead to deposition of excess of calcium salts in insoluble form. In witness of this, we may cite the calcareous deposits within tubercular areas; and, short of hard deposits, the visibility of smaller tubercular areas to X-rays is almost certainly associated with increased calcium percentage in such areas, as we have seen that it is in these renal calculi. Now in such areas the blood supply is very defective, and oxidation is undoubtedly embarrassed for want of oxygen. Also, in other areas, where structural change is occurring, due to diminished oxidation, we see a tendency towards calcification, as, for example, in arterio-sclerosis.

If the tendency to formation of renal calculi is associated with a gouty condition, as there appears to be good clinical evidence that it is, the large percentage of calcium as oxalate and phosphate in these stones, no less than the small amount of urate, leads to doubt as to whether uric acid is such an important factor in gout as has been generally believed, and further suggests some experimentation as to the composition of the

deposits of gout. One wonders whether these do consist so largely of insoluble urates, or if a careful examination would yield quantitative evidence of considerable amounts of calcium oxalate and phosphate. We have been on the outlook for 'tophi' and 'chalky knuckles,' but hitherto have not been able to find material; perhaps large gouty deposits are not so common as in the days of our forefathers.

In regard to treatment of the conditions leading to these renal calculi, the first thought which suggests itself is the elimination as far as possible of food rich in calcium from the diet. It must be pointed out, however, that although such an experiment would be well worth trying, yet calcium is in all our common foods, and if there is a strong tendency to diminished oxidation and oxalic acid production, there will always be enough calcium present to combine with such oxalic acid when produced. The only possible effect might be a slower rate of production on physico-chemical grounds if the calcium content of the plasma was lowered.

The richest food in calcium of all we consume is milk—although wines and, to a less extent, beers contain it in considerable amount. Now, milk in various forms enters so largely into the food of civilized man, that it would be almost as difficult wholly to exclude milk from a dietary as it is to shut the diabetic off from carbohydrates. We take milk not only directly as a drink, or mixed with other beverages, but in puddings, in biscuits, in various forms of baker's products, and lastly in cheese in all forms, either as cheese alone, or as a part of different dishes.

More success might attend an effort to get rid of the excess of calcium oxalate and phosphate by altering the reaction of the plasma and tissue-fluids.

It is extremely important to notice, however, that the therapeutic measures to this end must be the very diametrically opposite of those employed for uric acid, and if in any gouty case the trouble is a tendency towards calcium oxalate or phosphate deposition, as it sometimes may be, the usual anti-acid treatment is the very opposite of what is indicated.

Uric acid and its acid salts are soluble in alkaline media and insoluble in acid; the exact opposite holds for calcium oxalate and calcium phosphate, which are deposited in alkali and dissolved by acids.

The experimental evidence set forth in our analyses of the calculi is quite sufficient to show that there is no very great hope of dissolving calculi again by any diminished alkalinity which might be induced even

by vigorous therapeutic measures; for even the powdered calculi, as a rule, dissolve only slowly even in 1 in 4 hydrochloric acid.

This is not, however, the real clinical problem involved in such cases, but rather that of preventing growth and accretion of the calculi, and we would suggest that where such calculi have been obtained by operation, or otherwise, and have been shown by analysis to be calcium oxalate or phosphate, that certainly no ant-acid treatment should be recommended afterwards, as this will lead to more rapid growth, or to increased chance of growth, of such calculi, and it would be advisable to try a course of acid treatment.

For these reasons, a renal or vesical calculus should always be subjected to a rigorous chemical examination which may be the source of some clue, first, as to its origin, and, secondly, as to the nature of the disordered metabolism which has led to its formation.

Our thanks are due to Mr. Thurstan Holland for the radiographic observations and notes we have recorded and for the materials of calculi; and to Mr. Keith Monsarrat for the prostatic calculus described as Calculus XXIV.

THE GROWTH OF VARIOUS SPECIES OF BACTERIA AND OTHER MICRO-ORGANISMS IN ATMOSPHERES ENRICHED WITH OXYGEN[1]

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In a previous paper,[2] we have described experiments showing that the *Bacillus tuberculosis* will only grow between certain percentages of oxygen in the atmosphere to which it is exposed, and is killed either by absence of oxygen or by over eighty per cent. of oxygen. In other words, a partial pressure of oxygen of about 600 mm. of mercury is fatal to *Bacillus tuberculosis* equally with a very low pressure.

It was also found that such a high oxygen pressure stopped the growth of the organism *Staphylococcus pyogenes aureus,* and usually considerably inhibited, or stopped in some cases, that of *S. albus;* the third organism of this group, *S. citreus,* was less obviously affected.

Only twice have we obtained a fair growth of *S. aureus* in increased oxygen; as a rule, the contrast between the control tubes grown in air and the oxygen-grown tubes is most striking, the oxygen-grown tubes appearing to be quite sterile, while the others show a luxuriant growth.

There was this important difference, however, that the *B. tuberculosis* was killed outright by the oxygen, the small patches of started growths having turned dark brown at the end of the more prolonged period of about two to three weeks necessary to test the culture of this more slowly growing organism, while the controls were white and flourishing. On the other hand, at the end of the shorter period of one to two days necessary to test the culture of the Staphylococci, although the oxygen tubes had not grown, if they were now transferred to ordinary air a fairly good growth was obtained.

In the former paper a fair number of other organisms were similarly tested, such as *B. coli, B. typhosus, B. pyocyaneus, B. dysenteriae*

1. Towards the expenses of this research a Grant was made by the British Medical Association.

2. This Journal, Vol. IV, p. 177, 1909. An important erratum occurs in this paper. At p. 183, line 21, transpose the words 'oxygen' and 'air.'

(Flexner), *B. dysenteriae* (Shiga), *B. dysenteriae* (Kruse), *B. diphtheriae*, *V. cholerae*; and it was found that these were practically not affected by the higher oxygen percentages.

Since this aspect of the subject, namely, the effects of increased oxygen has not been nearly as much investigated as the opposite one of defect of oxygen, which from the point of view of classification of organisms into anaerobic and aerobic organisms has had a considerable amount of labour bestowed upon it by many investigators, we have carried our previous experiments somewhat further and tested the behaviour of our full stock of laboratory organisms toward increased amounts of oxygen in the atmosphere in which the organisms are grown.

The only other organism in our list specially affected by the increased oxygen percentage was *Bacillus pestis*, the growth of which we found to be invariably arrested by over 60 per cent. of oxygen in the atmosphere.

The *Bacillus pestis* is not killed by the oxygen at least after two or three days' exposure—we have not followed it further than this time interval—for, if the tubes which have not grown at all in the oxygen, while their fellows in air have grown quite well, be now removed to air, they commence growing and grow quite well.

That the *B. pestis* should behave somewhat similarly in regard to oxygen habits to the *B. tuberculosis*, is somewhat interesting in view of the occurrence of both plague and tuberculosis in ill-ventilated dwellings. If we add to these two organisms the Staphylococcic group, affected in somewhat lessened degree, we have the short list of what we might perhaps term the *oxyphobic* organisms from a fairly long list which we have tried to grow under similar and otherwise favourable conditions in air and in increased oxygen percentage respectively.

The complete list of the organisms which we have attempted to test in this way is as follows:—

1. *Bacillus tuberculosis* (human, bovine, and avian).
2. *Bacillus coli communis.*
3. *Bacillus typhosus.*
4. *Bacillus dysenteriae* (Flexner).
5. *Bacillus dysenteriae* (Kruse).
6. *Bacillus dysenteriae* (Shiga).
7. *Bacillus mallei.*
8. *Bacillus gaertner.*
9. *Bacillus prodigiosus.*
10. *Bacillus liquefaciens fluorescens.*
11. *Bacillus pneumoniae.*
12. *Bacillus pyocyaneus.*
13. Bacillus of hog cholera.
14. *Bacillus diphtheriae.*
15. *Bacillus anthracis.*
16. *Bacillus metchnikoff.*
17. *Bacillus megatherium.*
18. *Bacillus pestis.*
19. *Staphylococcus pyogenes aureus.*
20. *Staphylococcus pyogenes albus.*
21. *Staphylococcus pyogenes citreus.*
22. *Vibrio cholerae.*
23. *Sarcina lutea.*
24. *Sarcina aurentiaca.*
25. *Oidium albicans.*
26. *Proteus vulgaris.*

The organisms were sown upon slants of agar plugged in the usual way with cotton wool. Both the tubes intended to be grown in higher amounts of oxygen, and those in ordinary air, were placed in beakers standing in two bell-jars of similar size, and of about 3 litres capacity. Each bell-jar had a tubulure at the top, fitted with a rubber cork pierced by two glass tubes provided with stop-cocks, by means of which the bell-jar could be exhausted and oxygen afterwards allowed in. Each bell-jar had a ground flanged bottom or rim fitting on to a ground glass plate in the ordinary way, and the grinding was most accurate to avoid leakage over the lengthy periods of experimentation. Further, to avoid leakage, and so lowering of the oxygen pressure, a mound of putty was constructed of about one inch wider diameter than the base of the bell-jar, and mercury was run into this annular space so as to make a mercury seal; but no mercury was in the air-space within the bell-jar. In the case of the organisms to be grown in air, the bell-jar and other fittings were only set up as a dummy of the other experiment so as to make physical conditions of ventilation, air movement, drying, &c., alike for both sets of tubes, but the atmosphere within was not altered in any way. The amount of oxygen in the other bell-jar to contain the tubes to be grown in oxygen was enriched by pumping out the air by means of a water pump, and then admitting oxygen from a reservoir of that gas. When a high percentage of oxygen was required, the exhaustion and re-filling with oxygen was repeated several times. The oxygen used was manufactured in the laboratory from potassium permanganate. The atmosphere in the bell-jar was analysed, usually for oxygen and carbon-dioxide, at the beginning and end of the experiment. The percentage of carbon-dioxide was kept down very low by a flat dish of soda-lime included with the tubes under each bell-jar.

Description of Experiments

The numbering of experiments is carried on from the previous paper in which Experiments I to XV are recorded.

Experiment XVI.—The increased oxygen percentage bell-jar contained at the end of the experiment 50·7 per cent. of oxygen; controls in atmospheric air as above described. Two tubes of each organism in oxygen bell-jar and in air bell-jar.

Organisms experimented with were *B. coli, B. typhosus, B. diphtheriae, B. dysenteriae* (Flexner, Kruse, Shiga), *S. albus, S. aureus, S. citreus.*

Duration of experiment about thirty hours.

RESULTS.—*B. coli, B. typhosus, B. diphtheriae* showed no differences as between air and oxygen; as also *S. citreus* and *B. dysenteriae* (Flexner); there was possibly slight inhibition of growth in the cases of *B. dysenteriae* (Shiga) and *B. dysenteriae* (Kruse). The two Staphylococci *S. aureus* and *S. albus*, however, showed good growths in the air-grown controls, and no growth whatever in the oxygen-grown tubes.

EXPERIMENT XVII.—Oxygen percentage at end 50, the bell-jar having been partially evacuated and charged only once at beginning. Duration of experiment about fifty hours. Organisms used were *S. aureus* and *S. albus*, and *B. dysenteriae* (Kruse and Shiga), two tubes of each in air and in increased oxygen.

RESULTS.—All the controls in air had grown well. The Kruse and Shiga in oxygen had also grown well; but the two Staphylococci, *aureus* and *alba*, had not grown in the least.

EXPERIMENT XVIII.—Bell-jar partially exhausted and re-filled with oxygen for four times; final percentage of oxygen was 84 per cent.

The following organisms were used, two tubes each in air control and in the oxygen, viz.:—*B. mallei, B. gaertner, B. prodigiosus, M. agilis, B. pneumoniae, B. pyocyaneus*, Hog Cholera, *Sarcina lutea, Sarcina aurentiaca, Oidium albicans, Proteus vulgaris.*

The controls in this case were grown in the same incubator, but not under a bell-jar.

Duration of the experiment about forty-five hours.

RESULTS.—No differences were observable in any of these organisms between amount of growth in oxygen and that in air.

EXPERIMENT XIX.—Oxygen percentage raised to 77 per cent.; two tubes of each organism in usual controls, and two in oxygen. Organisms tested:—*B. anthracis, B. metchnikoff, B. megatherium*, and *B. diphtheriae*. Duration about forty-eight hours.

RESULTS.—Equal growths in all cases in air and in oxygen.

EXPERIMENT XX.—The two Staphylococci were here grown in a lower percentage of oxygen (but still higher than air) to attempt to settle lower limit of oxygen inhibition. Richness of oxygen was 45 per cent., duration of experiment about seventy hours. Two tubes each in air control and in the oxygen mixture.

RESULTS.—*Staphylococcus albus*: Both controls show good growth; one oxygen tube shows good growth, the other a poor growth.

Staphylococcus aureus: Both controls show poor growth; one oxygen tube shows poor growth, the other shows hardly any growth at all.

EXPERIMENT XXI.—Oxygen percentage 91; controls as usual, two tubes in each lot. Duration of experiment about three days. Organisms used: *Bacillus pestis* and *Vibrio finkler* (Prior).

RESULTS.—*Bacillus pestis*: The two controls in air show fair growths; *the two oxygen tubes show no growth*. *V. finkler* (Prior): All four tubes show good growth.

EXPERIMENT XXII.—Oxygen percentage 90; controls as usual; two tubes in oxygen and two in air. *Bacillus pestis* used only; duration two days.

RESULTS.—Both controls show good growth; neither of the two oxygen tubes show any growth at all.

EXPERIMENT XXIII.—Oxygen percentage 75; controls as usual, three tubes in air and three in oxygen. The organism used was a strain of Streptococcus. Duration of experiment two days.

RESULTS.—All six tubes show a good growth; there may just be a slight difference in favour of the air-grown tubes.

EXPERIMENT XXIV.—Oxygen percentage 93; controls as usual; two tubes of each organism in oxygen, and two in air. Organisms tested: *Bacillus pestis* and above strain of *Streptococcus*. Duration of experiment two days.

RESULTS.—All four tubes of *Streptococcus* show practically equal growth. The two air-grown tubes of *Bacillus pestis* show good growth; the two oxygen-grown tubes show no growth.

EXPERIMENT XXV.—Oxygen percentage intermediate at about 60 per cent. to test the sensitive organisms for range; two tubes each in oxygen and in air. Organisms tested: *Bacillus pestis*, *Staphylococcus albus* and *Staphylococcus aureus*. Duration of experiment three days.

RESULTS.—*Bacillus pestis*: The two oxygen tubes show no growth; the two air tubes show a good growth. *Staphylococcus albus*: The two controls show a good growth; the oxygen tubes (60 per cent.) show a fair growth, roughly about half as great as the air tubes. *Staphylococcus aureus*: A similar result to the *albus*.

In order to test whether the plague organism was killed by the increased oxygen, or only inhibited in its growth, the two tubes previously in oxygen, and showing no growth, were now placed in the

incubator for two further days in air, when a good growth appeared, showing that the organism had only been inhibited and not killed by the previous sojourn in oxygen (60 per cent.).

EXPERIMENT XXVI.—Oxygen percentage at 78·4. Tubes of *Bacillus pestis* and of *Staphylococcus albus*, two each in air and in oxygen. Kept for two days.

RESULTS.—*Bacillus pestis*: Controls show good growth; oxygen tubes show not a trace of growth. *Staphylococcus albus*: Controls show excellent growth, and oxygen tubes just a trace of growth.

All four of the oxygen tubes were now taken and placed in the ordinary way in air in the incubator. At the end of two days all four had grown normally equally with previous growth of the controls. Hence, the growth had only been kept in abeyance by the oxygen.

EXPERIMENT XXVII.—Oxygen percentage 88; two tubes each of *B. pestis*, *S. albus* and *S. aureus*, in air and in oxygen respectively. Duration three days.

RESULTS.—*B. pestis*: Controls in air show good growth; oxygen tubes no growth. *S. albus*: The oxygen tubes do show a distinct growth, but not to be compared with air-grown controls. *S. aureus*: The oxygen tubes show a growth nearly as good as control. Accordingly, this experiment, like the next, gives an exceptional result with *S. aureus* in oxygen. *Bacillus pestis*, however, uniformly refuses to grow in oxygen throughout, although tried on seven occasions with two tubes on each occasion.

As before, the negative tubes grew when subsequently tried in air.

EXPERIMENT XXVIII.—Oxygen percentage 86. Two tubes of *Bacillus pestis*, *Staphylococcus aureus*, and *Staphylococcus albus*, respectively, in oxygen, and two tubes of each organism in air. Duration of experiment two days.

RESULTS.—All controls show good growth. The plague organism, as usual, has not grown at all in the oxygen; the *Staphylococci*, both *albus* and *aureus*, do show a fair growth, even in the oxygen, but not so luxuriant as in the air.

CONCLUSIONS

1. A list of twenty-six organisms has been tested as to comparative growth in atmospheres of air, and of air enriched in high degree by oxygen.

2. Two organisms stand out prominently as oxyphobic, namely, those of tuberculosis and of plague, and the only others affected adversely by the increased oxygen are the organisms of the Staphylococcic group, particularly *Staphylococcus pyogenes aureus* and *Staphylococcus pyogenes albus*.

3. The *Bacillus tuberculosis* is not only arrested in growth, but is killed by the three weeks exposure to the higher percentage of oxygen necessary to determine the question of comparative growth. The *Bacillus pestis*, although it uniformly refused to grow on seven occasions with percentages of oxygen of 91, 90, 93, 60, 78, 88, and 86, respectively, was not killed by these amounts during the two to three days demanded for the decision of the experiment, since the tubes grew afterwards when brought into air cultivation. It has not been determined whether longer exposure for two or three weeks would kill the organism.

A NOTE ON PURINE METABOLISM IN HIBERNATING ANIMALS

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An increased output of purine compounds has been observed in man in various conditions which are associated with increased production of heat.

Cathcart and Leathes[1] showed that exposure to cold produced a rise in the amount of endogenous uric acid excreted. The subject of their experiments stripped and remained at rest for between two and three hours, the skin being kept wet by frequent immersion in cold water. The rectal temperature was by these means reduced about 0·5°. The output of uric acid rose above the normal level during the exposure to cold, and remained above it throughout the ensuing twenty-four hours, the amount excreted during this time being in one experiment 80 per cent. greater than the average.

Again, in conditions of fever, the excretion of large amounts of purine bases,[2] and of uric acid,[3, 4] has been observed.

The increased output of purines in these cases might have been due (1) to liberation of purines from the nucleo-proteins of the tissues; (2) to

a synthesis of purines; (3) to diminished destruction of uric acid. Recent experiments on the destruction of uric acid by human tissues *in vitro* have, however, given negative results.[5] Analyses of urine do not allow one to decide between these three possibilities. Dr. Pembrey suggested that the question might be investigated further in hibernating animals. During the awakening from hibernation a very rapid rise of body temperature (e.g., of 19° in forty-two minutes) takes place.[6] The dependence of this rise of temperature upon increased production of heat is shown by the fact that the respiratory exchange is greatly increased, in some cases about a hundred fold, at the time of awakening; moreover, on the assumption that the substance oxidised at this time is fat, it has been calculated that the combustion indicated by the respiratory exchange would provide amply for the observed heating of the body.[6] Such animals, therefore, provide material for ascertaining whether any change in the total purine content of the body is associated with an increase in the production of heat.

Dormice were used for these experiments. A large number were obtained in the autumn, and were fed on grain, carrots, chestnuts and apples. The estimations of purines were made by the method of Burian and Walker Hall.[7] The intention was to make duplicate estimations (1) before, (2) during, hibernation; (3) during, (4) after, awakening; but the high death rate allowed only five estimations, involving a total of fifty dormice, to be made. However, the close agreement of the results seems to make them worth consideration. No attempt was made to obtain a further supply of dormice in the spring, as it was desirable that all should have been kept under the same conditions throughout. Owing to the exceptionally mild winter, the animals never remained torpid for more than a few days at a time. At the end of February, therefore, when there seemed to be no prospect of a period of natural hibernation, the cages were placed in a cooled chest (temp. 5°); the animals became torpid during the same day, and were allowed to remain in this state for four days. The body temperature, taken within the coil formed by the rolled-up body, was at this time from 6·3° to 8·2°; the reading obtained in this way is 1° to 2° lower than the rectal temperature.[6] Those which were to be awakened were taken to a warm room; in about an hour they were quite active, the body temperature being then from 22° to 34° They were killed within two hours of removal from the cooled chest.

	Number of mice	Weight of mice	Mean weight of mouse	Weight of tissue after mincing	Purine Nitrogen		
					Total	Per cent. of tissue	Per mouse
		g.	g.	g.	mg.		mg.
Before Hibernation							
October 11th	12	171·5*	14·3	165·5	126·7	0·077	10·58
November 1st	12	171·5*	14·3	166·2	138·7	0·083	11·58
Hibernating							
March 5th	10	136·1	13·6	133·2	109·1	0·081	10·9
Waking							
March 1st—A	8	121·1	15·1	116·9	125·16	0·107	15·65
March 1st—B	8	118·7	14·8	114·2	118·30	0·104	14·79

The percentage of purine nitrogen found in the waking dormice is over 30 per cent. higher than that found before or during hibernation. This difference cannot be attributed to loss of fat during the winter, since those mice which were killed at the same time, but in the torpid state, show no increase in comparison with those killed in the autumn. Moreover, such a concentration of purines would not be produced even by the removal of all the fat in the body, since the greatest proportion of fat which has been found in autumn dormice of the weight of those under consideration is 17 per cent.[8]

Again, the difference observed cannot be due to the loss of body weight brought about at the time of awakening by the increased discharge of water and carbon dioxide. Pembrey's numerous observations show that a dormouse loses, on an average, less than 1 per cent. of its weight within one and a half hours of the commencement of awakening.[6]

Nor can the result be attributed to an arrest of purine destruction at the time of awaking. If such arrest is to explain the increase of purine nitrogen from, presumably, 0·08 per cent. to 0·105 per cent. within two hours of arousal, the hibernating animal must have been converting into non-purine compounds at least 15 per cent. of its purines per hour; in view of the fasting condition and greatly reduced respiratory exchange in hibernation this seems altogether unlikely. The increased amount of purines appears, then, to be due to a synthesis of these compounds during the return of the animal to the active state.

* At this time there was a sufficient number available to allow of the selection of series having the same total weight

As the increase observed is brought about within one or two hours, some or all of the newly formed purine is presumably at this time in a free state. An attempt was made in the first analysis to estimate the free and combined purines separately,[9] but the method of separation did not appear satisfactory.

It is possible that the additional amount of purines in the body plays a part in stimulating the production of heat. The administration of xanthin and caffein by the mouth produces a rise of body temperature.[10]

The estimations recorded here give, of course, no indication as to the tissue in which the formation of purines occurs. The heat liberated at the time of awaking is held by most workers on hibernation to come chiefly from the muscles,[11] and there is some evidence that muscle is capable of synthesising purine compounds. This evidence may be summarised as follows:—(1) Adult muscle differs from other tissues in containing a percentage of hypoxanthin which is very large in proportion to the amounts of adenin and guanin present;[12] this is true even of animals (e.g., the dog) in which the muscles contain no adenase;[13] (2) the perfused hind limbs of the dog eliminate uric acid continuously while maintaining a constant purine base content.[14]

Summary

Dormice when awaking from hibernation were found to contain 30 per cent. more purine nitrogen than was present before or during hibernation. The results indicate that a synthesis of purines takes place at the time of awakening.

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THE ALKALOID OF PITURI OBTAINED FROM *DUBOISIA HOPWOODII*

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I was recently brought a quantity of material consisting of fragments of twig and lanceolate leaves, and of a distinctly pleasant aromatic smell, with the request that I would undertake its investigation.

Those interested in it maintained that it had a wonderful influence, when smoked, of curing the pain of neuralgia, and at the same time producing a feeling of exhilaration.

I pointed out that it was desirable, before undertaking any work upon it, to have it identified botanically, partly suggesting what the active principle might be, but more particularly to safeguard against entering upon a big piece of work in an already investigated field. No information was available, however, nor could it be obtained within the month, and the work was undertaken meanwhile with the object of furnishing a preliminary report. A very short space of time was required to show the presence of an alkaloid, and an alkaloid of a very poisonous character. Further, it was apparent that the alkaloid was volatile, appearing in steam distillates, and in the smoke from the burning material.

Action on man. In the first place the fine dust from the broken material, when caught in the nose acts like snuff, causing a powerful desire to sneeze.

When smoked (which can only be done by frequently applying a lighted match to the pipe bowl) there is no great effect. The pulse is somewhat slowed, and after a half pipeful or so in my own case I had usually had enough and subsequently felt heavy-headed. I am as a rule a non-smoker, but after smoking the herb my sleep at night was less deep than usual, a result which with me also follows the smoking of tobacco. An after effect of smoking the material was a feeling that something was stuck in the oesophagus. This has followed the smoking on two separate occasions, and the unpleasant feeling has persisted for a day. Inhalation

of steam saturated with the hydrochloride of the alkaloid caused marked nausea.

Action on the Frog. The alkaloid caused death in a few minutes after injection into the dorsal lymph sac.

The frog passes into a condition of catalepsy without power of movement, and it is hard to say when death occurs. The heart on exposure half an hour later is still beating.

In smaller doses than the lethal, the paralysis sets in, and the frog when turned on its back cannot correct its position. Recovery occurs in from 20 to 40 minutes.

The same action on the frog was obtained with all the following preparations:—(*a*) The steam distillates, (*b*) the smoke collected in physiological saline solution (0·9 per cent. sodium chloride), and (*c*) an alkaloidal extract made with 0·2 per cent. hydrochloric acid, and subsequent purification by extraction with ether after rendering alkaline, and re-extraction from the ether by 0·2 per cent. hydrochloric acid.

In greater detail an injection causes a twitching of isolated muscles throughout the frog's body, then a sluggish response to stimuli so that an outstretched hind leg is only very slowly drawn up on pinching the toe, and finally complete absence of response, and paralysis within five minutes.

There is a loss of the bright green pigmentation of the skin, which takes on a darker dusky appearance.

Action on Frog's Heart. This is very characteristic. (i) A drop of solution containing the alkaloid caused, when applied to the heart externally, a cessation of beat in profound diastole. After 17 beats had been missed in this condition there came a systole, then another long diastolic interval and another isolated systole. This continues for some time, the diastolic standstills becoming of gradually less and less duration, until a slow powerful beat is established which gradually returns to the normal rate and with the former magnitude.

(ii) Further applications are now incapable of producing a new diastolic standstill. All the effect they have is to produce a slight slowing of the beat, with the accompanying increase in magnitude (fig. I).

This action of the drug upon the frog's heart is very characteristic.

(iii) If the heart be first painted with *Atropine* and the drug then applied the only effect obtainable is that of slight slowing, as in No. ii above. Consequently it may be stated that the alkaloid first stimulates

the vagus ' endings ' causing prolonged diastole, and then paralyses them (escape of the heart from the diastole).

There is left, however, an action upon the heart muscle direct, which can be obtained by fresh application of the drug and after atropine.

Action on Nerve Trunks. No effect could be noted on excitability or conductivity when the nerve trunk of an ordinary sciatic-gastrocnemius preparation of the frog was employed. No elaborate apparatus was used, however.

Action on Nerve endings in Skeletal Muscle. The classical experiment for testing the action of curare on the frog was employed. The alkaloid was injected into the lymph sac of a frog, one of whose legs (the right) was protected from the drug by a tight ligature passing under the sciatic nerve and around the thigh.

Both legs were then skinned, and the sciatic of the left leg also exposed, and this and the right sciatic nerve stimulated alternately with tetanic shocks. After a time there was a loss of response in the left leg, but not in the right leg (which was protected by the ligature).

The alkaloid thus paralyses motor nerve endings, for a response can still be obtained by stimulating the muscles directly.

Action on Mammals. Rabbit. The doses administered proved fatal very rapidly. The animal was convulsed, the convulsions being of the clonic type, and death was apparently due to failure of respiration, for the heart could be seen beating when only a snatched and shallow respiration was occurring at long intervals.

Isolated Rabbit's Heart. A perfusion of the heart with oxygenated Ringer's Solution was performed according to Locke and Rosenheim.[1] The heart was beating very poorly indeed, but owing to several previous failures it was decided to make an attempt to perfuse the alkaloid. A couple of cubic centimetres of a purified extract were added to the circulating Ringer solution with an astounding result. At first the heart was slowed but showed beats of increasing amplitude. Suddenly it escaped from the vagus control and commenced beating at an excessive pace and with tremendous vigour. So great was the pace that a period followed in which beats kept being dropped with a consequent beat of very great excursion. This continued for some time, and then the heart slowly quietened down again (see fig. II).

Dog. A small dose injected subcutaneously caused a very frisky

1. Locke and Rosenheim, *Journal of Physiology*, Vol. XXXVI, p. 205.

terrier to become unsteady when balancing on his hind legs, then to quieten down, and finally to retch. Vomiting did not occur, however.

Action on Blood Pressure. A series of blood pressure tracings were taken. Morphine, chloroform and ether, and chloroform, were administered. The vagi were divided in the earlier experiments, not in those with curare. The drug was injected into the jugular vein and immediately gave rise to convulsions (fig. III). In later experiments curare was therefore used.

An injection is accompanied by a marked rise in blood pressure, with vaso-constriction as shown by an intestinal plethysmograph. The heart is slowed, and the magnitude of the beat increased, as the result of the first injection, but in a very short interval of time the beat becomes extremely rapid. Subsequent injections are (if the first injection was strong) without a renewal of the slowing effect. The renewed rise of blood pressure with each fresh injection of the alkaloid is due to the marked vaso-constriction in the first place (see figs. V and VI).

The plethysmograph lever, however, shows a rise with the continued high blood pressure. Careful analysis points to the vaso-constriction period corresponding with the slowing of the heart period. The heart then becomes very rapid, and this is the time when the plethysmograph tracing rises. Owing to a fault in the plethysmograph the excursions of the lever were only about half what they should have been.

The above pharmacology is very suggestive of nicotine, and in no single point is any contradiction apparent. Further, the volatility of the alkaloid is also in agreement.

The chemistry of the alkaloid was therefore investigated, and the conclusion arrived at that the alkaloid is indeed nicotine. At this point a study of Kobert's *Lehrbuch der Intoxikationen*, page 1071, led to a true recognition of the nature of the material I was handling.

Under Nicotine is to be found a reference to Pituri (*Duboisia hopwoodii*) v. Muller, 'Pituri . . . welches wie ein Gemisch von Kautabak und Kokablättern wirken soll. Man vermutet, dass, das darin enthaltene Alkaloid "*Piturin*," mit Nikotin verwandt, oder identisch ist.'

Immediately, some of the material was handed to Professor Ewart for comparison with specimens of *Duboisia hopwoodii* in the Melbourne Herbarium. The reply was an absolute confirmation of their identity. A short historical account of the previous work on the alkaloid of Pituri (*Duboisia hopwoodii*) will now be given.

The use of Pituri by the natives of Queensland and the Northern Territory has been described and is still described by those who come into contact with the far back tribes.

The first to describe its pharmacological action was Bancroft,[1] who, however, does not give any suggestion of the true nature of the alkaloid contained.

Ringer and Murrell[2] next had the opportunity of examining a small amount of the alkaloid as regards its action on the cat, and they discuss its possible relationship and intermediate position between atropine on the one hand, pilocarpine and muscarine on the other.

Fraser[3] considers that the alkaloid is not nicotine owing to a difference in action on the pupil. He describes the effect on the blood pressure, the increased systole of the heart, and the vagus effects, as similar to the effects produced by nicotine.

Langley and Dickenson[4] give a most comprehensive account of the pharmacology, and sum up their experiments with the clear and definite statement that ' Its physiological action is identical with nicotine.'

From the chemical side Petit[5] identified the alkaloid as nicotine on the following evidence:—(*a*) Rotation in alcohol, — 123·9; for nicotine (Buignet), — 121·9. (*b*) Power of neutralising sulphuric acid. (*c*) One analysis of the platinochloride, Pt 34 per cent., Cl 36 per cent. (*d*) Evidence of a qualitative nature.

Liversidge considered the work of Petit inconclusive. As the result of numerous analyses of the platinochloride, he shows that there is no fixed composition. The platinum varied between 34·15 per cent. and 38·40 per cent., while the chlorine varied between 31·32 per cent. and 36·86 per cent. He concludes, as the result of combustion analysis, that the alkaloid has the formula $C_{12}H_{16}N_2$, and not $C_{10}H_{14}N_2$ (nicotine). It will thus be seen that in spite of much previous work the exact nature of the alkaloid is still in doubt.

My own investigations are now published as an independent confirmation of much that has already been done, and as proof, especially on the chemical side, *that the alkaloid of pituri is nicotine.* It agrees with nicotine in the following points:—(1) Its pharmacology;

1. Bancroft, *Journal Queensland Phil. Soc.*, March, 1872.
2. Ringer and Murrell. *Journal of Physiology*, Vol. I, p. 377, 1879.
3. Fraser, *Proc. Roy. Soc. Edinburgh*, p. 200, 1879.
4. Langley and Dickenson, *Journal of Physiology*, Vol. XI, p. 265, 1890.
5. Petit *Pharmaceutical Journal*, Vol. IX, p. 819. 1878-1879.

(2) its volatility; (3) the positive iodo-nicotine test; (4) the solubility in water, and more particularly in the way an opalescent warm solution will clear on cooling; (5) its unwillingness to yield crystalline salts, e.g., the hydrochloride and sulphate.

In an early attempt to prepare the hydrochloride it was noticed how on evaporation the alkaloidal salt was carried over in the steam, and it is an observation which has been made with nicotine that the hydrochloride is more volatile than the free base. The hydrochloride was left on evaporation as a thick brown syrup, which after a week in a vacuum over strong sulphuric acid showed star clusters of long radiating needles. When removed from the desiccator, water was absorbed and the crystals re-dissolved.

It may here be mentioned that several times on adding strong hydrochloric acid to the watery solutions of the free alkaloid a fine red colour developed fairly rapidly. No characteristic absorption bands were shown, only general absorption of the violet, blue and green.

Schwebel[1] mentions the same reaction in connection with nicotine. (6) *The oxalate* was prepared by adding an ethereal solution of the free base to an ethereal solution of oxalic acid. The precipitate was re-dissolved in a little warm 96 per cent. alcohol, and ether added until a fine opalescence was formed. Pearly crystal plates separated on standing. M.Pt. 77° C. becoming 109°-111° after re-solidifying; decomposition, 115° C.[2]

(7) *The platino chlorides.* Two have been prepared, and correspond to those of nicotine.

(*a*) *Orange salt* crystallising beautifully from solutions containing excess of hydrochloric acid. Absolute identity of crystalline form with the same salt prepared from nicotine tartrate (Merck).

(*b*) *Yellow salt.* Generally obtained as a light yellow powdery deposit after the separation of (*a*) and when the excess of hydrochloric acid has somewhat diminished.

According to the dictionaries of Organic Chemistry—Beilstein's 'Handbuch der Organischen Chemie' and Fehling's 'Neues Handworterbuch'—(*a*) would have the formula $C_{10}H_{14}N_2$, 4HCl, $PtCl_2$; (*b*) the formula $C_{10}H_{14}N_2$, 2HCl, $PtCl_2$, in the case of nicotine.

The percentages of platinum and chlorine would be:—for (*a*)

1. Schwebel, *Ber. Deut. Chem. Ges.*, Vol. XV, p. 2850.
2. Parenty and Grasset, *Bull. Soc. Chem. Paris*, Vol. XIII, p. 299.

Nicotine, 4HCl $PtCl_2$, platinum 33·91 per cent., chlorine 37·18 per cent.; for (*b*) Nicotine, 2HCl $PtCl_2$, platinum 38·8 per cent., chlorine 28·4 per cent.

A third platino-chloride has:—Nicotine, 2HCl $PtCl_4$, platinum 34·03 per cent., chlorine 37·30 per cent.

The following analyses were made on the platino-chlorides. A pure yellow salt was not obtained in sufficient quantity for analysis, but always had some orange salt admixed:—

Preparation		*Calculated Mol. Wt. of Base* Minus x hydrogen atoms
(1) Yellow (*b*), first preparation well washed, but not recrystallized	Platinum 36·02 % Chlorine 32·2 %	172
(2) Yellow (*b*), a distinct preparation from (1)	Platinum 36·08 % Chlorine 32·29 %	172
(3) Yellow (*b*), completely independent preparation to (1) or (2)	Platinum 35·79 % Chlorine 32·07 %	174
(4) *Pink Salt*, identical with orange salt (*a*), but coloured by the red HCl reaction previously described	Platinum 34·25 % Chlorine 36·1 %	168
(5) *Orange Salt* (*a*), a lovely sample beautifully crystallized	Platinum 34·46 % Chlorine 34·60 %	174
(6) Orange Salt (*a*), lovely crystals	Platinum 33·76 % Chlorine 36·52 %	171·6
(7) Pure orange nicotine platinum chloride from nicotine tartrate (Merck)	Platinum 34·06 % Chlorine 35·72 %	172
(8) *Orange nicotine platinochloride from nicotine tartrate (Merck)	Platinum 33·70 % Chlorine 35·96 %	177

**Note.*—The actual amount of nicotine platinochloride analysed in this case was only 0·18 gram. Hence the slightest experimental losses are magnified in the calculated Mol. Wt. of base.

The figures for the Mol. Wt. of base are obtained by calculating the amount of platinochloride and chlorine corresponding to one atom of platinum and then subtracting the platinum and chlorine from the weight of platinochloride so obtained.

Examples from (6):—

Platinochloride		*Platinum*		*Chlorine*
0·3729 gram		0·1256 gram		0·1362 gram
576·6 salt	<———	194·3 platinum	———>	210·7 chlorine

576·6 − (194·3 + 210·7) = 171·6 the weight of base + x hydrogens.

The number of hydrogen atoms (x) will be between 2 and 4.

The above estimations were carried through by Wallach's[1] method

1. Wallach, *Ber. deut. Chem. Gesellsch.*, p. 753 (1881).

for the simultaneous estimation of platinum and chlorine in double platinochlorides.

The results show the platinum tending to be slightly on the high side, the chlorine not inconsiderably on the low side, as required by theory. Petit and Liversidge's analyses show the same error, which, as Liversidge suggests, may be due to the platinochlorides not showing a constant composition.

Refractive Index of Alkaloid. This was obtained using a sample of alkaloid distilled in a current of hydrogen. The alkaloid passed over in a colourless condition at a temperature in the close neighbourhood of 240° C. The distillate in bulk had the faintest yellow tinge.

A Zeiss apparatus (for examination of a single drop) was employed.

Temperature		*Refractive Index*
28° C.	...	1·5220
25° C.	...	1·5240
20° C.	...	1·5265
16° C.	...	1·5275
13° C.	...	1·5290
10° C.	...	1·5305

This gives for 15° C. a refractive index 1·5283

For nicotine N_D = 1·52828 (Llandolt, *Ann. Chem.*, Vol. CLXXXIX, p. 317).

Optical Rotation. The free base showed a negative rotation. On carefully adding hydrochloric acid to a slight excess the rotation became positive. Thus the following figures were obtained:—

(1) *Base* Aq. + dilute HCl to double volume	1 dcm. tube	−	1·46	$\frac{1·46}{0·306}$	= 4·77
	2 dcm. tube	+	0·306		
(2) *Nicotine* Aq. + HCl to double volume	1 dcm. tube	−	1·63	$\frac{1·63}{0·354}$	= 4·60
	2 dcm. tube	+	0·354		
Temperature for (1) and (2), 23° C.					
(3) *Base* Aq. *Hydrochloride*	2 dcm. tube	−	1·38	$\frac{1·38}{0·27}$	= 5·11
		+	0·27		
(4) *Base* Aq. *Hydrochloride*	2 dcm. tube	−	2·497	$\frac{2·497}{0·475}$	= 5·26
		+	0·475		
(5) *Nicotine* Aq. *Hydrochloride*	2 dcm. tube	−	2·11	$\frac{2·11}{0·415}$	= 5·09
		+	0·415		

In (3), (4) and (5) the base was first examined in a two decimetre tube. Then a given volume was made acid with a few drops strong HCl + Aq to one-eleventh of its bulk, and re-examined as hydrochloride in

a 2·2 dcm. tube, i.e., equal to a 2 dcm. tube of the original base-solution. Temperature for (3) 23° C., for (4) and (5) 32° C.

It is further evidence of the identity of the base with nicotine when the expected readings for the hydrochloride, as obtained by calculation, are compared with those actually found. For the calculation the following figures were used:—

$a_D = -74{\cdot}13$ for very weak water solutions of nicotine[1]
$a_D = +14{\cdot}5$ for nicotine hydrochloride + 98 % water[2]

(1) *Base* Aq.	1 dcm. tube Rotn.	−	1·46			
Hydrochloride	Calculated	+	0·2875	Found	+	0·306
(3) *Base* Aq.	2 dcm. tube Rotn.	−	1·38			
Hydrochloride	Calculated	+	0·2697	Found	+	0·27
(4) *Base* Aq.	2 dcm. tube Rotn.	−	2·497			
Hydrochloride	Calculated	+	0·488	Found	+	0·475

In these experiments the direct comparisons with nicotine were made owing to the scant figures published for the rotation of nicotine and its hydrochloride in presence of water. A Schmidt and Haensch three shadow polarimeter was used, and the majority of the readings were sharp to within ± 0.01°, but with the longer tubes ± 0·02° was all the accuracy attainable. Averages of ten to twelve separate readings were then used.

Rotation of Pure Alkaloid (free base) *in water.* 0·6522 grms. of pure distilled base were dissolved in 25 c.c. distilled water and examined in a 2 dcm. tube at 21° C.

Rotn. 3·686°.

The reading was obtainable with accuracy, the solution being clear and colourless.

Translating for comparison with figures given by M. Popovici,[3] 1·3044 grms. free base in 50 c.c. water gave 221′ rotation in a 2 dcm. tube, which result exactly agrees (within the limits of experimental error) with Popovici's figures, which for 1·3044 grms. nicotine give 225′ (by interpolation), or 219′ (using the figure 1′ = 0·595 mgrm. nicotine).

Combustion Analysis. These were carried out on the pure base obtained as follows:—200 grms. of pituri were made alkaline with the addition of 50 c.c. of 40 per cent. sodium hydrate and 2 litres of water, and were distilled in steam till 4 litres of distillate had been collected.

1. Llandolt, *Ann. Chem.*, p. 317.
2. Schwebel, *Ber. deut. Chem. Ges.*, Vol. XV, p. 2850.
3. Popovici. *Zeit. Phys. Chem.*, Vol. XIII, p. 447.

The free base was fixed by oxalic acid, and the distillate then concentrated in vacuo.

It was now made alkaline, and as the alkaloid did not separate as an oil, it had to be extracted by repeated quantities of ether.

The ether was removed, and the residue distilled in hydrogen. 1st fraction distilled at 98° C. (chiefly water); 2nd fraction distilled about 240° C. The middle portions of this fraction were used for the combustions. Suitable amounts were immediately filled into small tubes, which were then sealed, and weighed, to be used in due course.

RESULTS

Found	*Pituri Body*			*Nicotine* $C_{10}H_{14}N_2$	*Calculated* for *Liversidge* $C_{12}H_{16}N_2$
Carbon	71·90 %	74·23 %	73·14 %	74·06 %	76·59 %
Hydrogen	8·56 %	8·60 %	9·02 %	8·64 %	8·51 %
Nitrogen	16·2 %	17·7 %	17·5 %	17·3 %	14·9 %

These results uphold the nicotine formula, and completely discount the formula suggested by Liversidge.

The Amount of Nicotine in Pituri. 20 grms. of the powdered material dried at 60° C. for two hours, and submitted to the usual process for nicotine estimations yielded 250 c.c. distillate requiring 31 c.c. $\frac{N}{10}H_2SO_4$ for neutralisation (Rosolic Acid as indicator); next 120 c.c. distillate requiring 2 c.c. $\frac{N}{10}H_2SO_4$; total, 33 c.c. $\frac{N}{10}H_2SO_4$ = 2·67 grms. nicotine per 100 grms. of pituri.

Throughout the experimental work evidence for the existence of another alkaloid has been distinctly absent.

After removal of the volatile nicotine there is no fixed alkaloid remaining (confirmation of Liversidge), and any other alkaloid present must therefore be volatile.

Until large quantities of the crude base can be fractionally distilled, there is no likelihood of such a possible alkaloid being separated.

The pharmacology and the analytical data indicate that such an alkaloid or alkaloids could only be present in small quantities, and even then would in all probability be closely allied to the nicotine. These statements are made on account of a belief, quite unsupported by any observations I have been able to make, that pituri has a peculiar 'cocaine-like' action. At all events, there is no doubt that *the alkaloid* of pituri (*Duboisia hopwoodii*) is *Nicotine*.

Summary

(1) The alkaloid of pituri is nicotine.

(2) No evidence is forthcoming that there is any exhilarating or cocaine-like action, or that there is any pharmacological action, other than what nicotine can produce.

(3) There is no fixed alkaloid.

(4) There is no evidence to suggest any other volatile alkaloid than nicotine, though small quantities of allied alkaloids, as in tobacco, may exist.

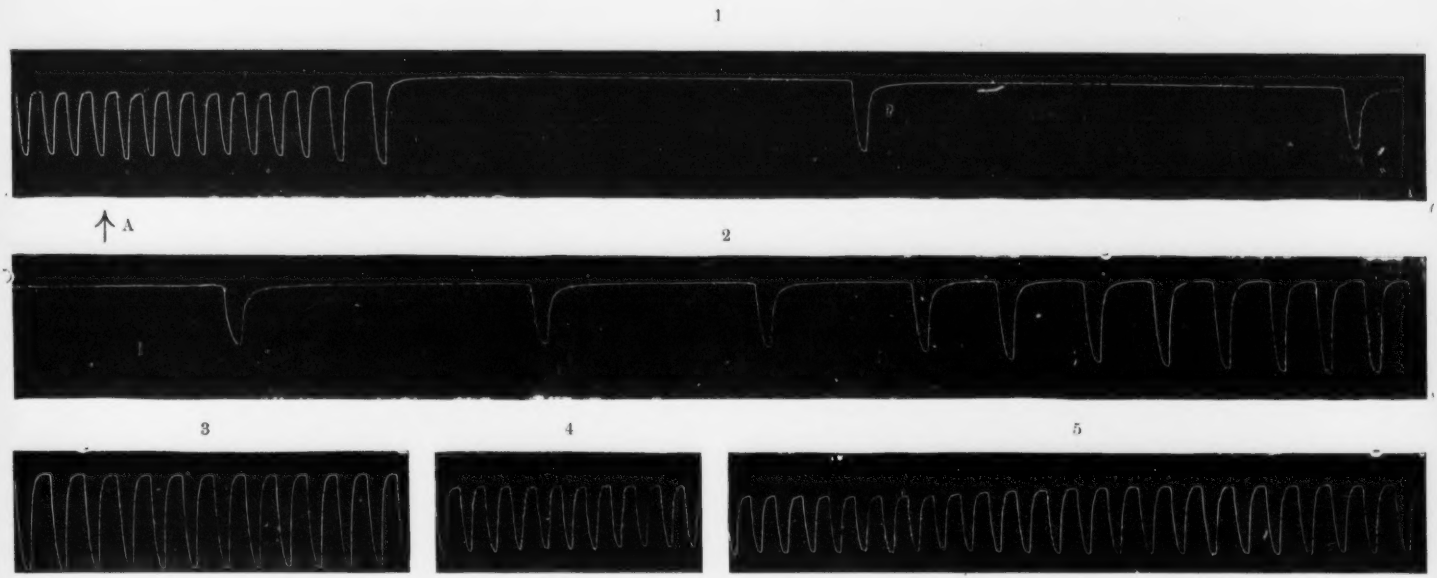


FIG. I. FROG'S HEART

Painted with dilute alkaloid hydrochloride solution at A. 1, 2 and 3, consecutive. Interval between 3 and 4, and between 4 and 5 = 50 beats of heart. At B, again painted heart with the alkaloid.

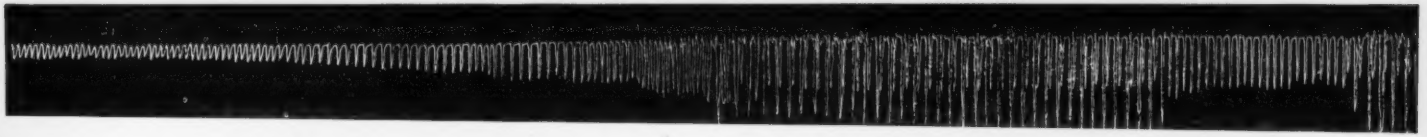
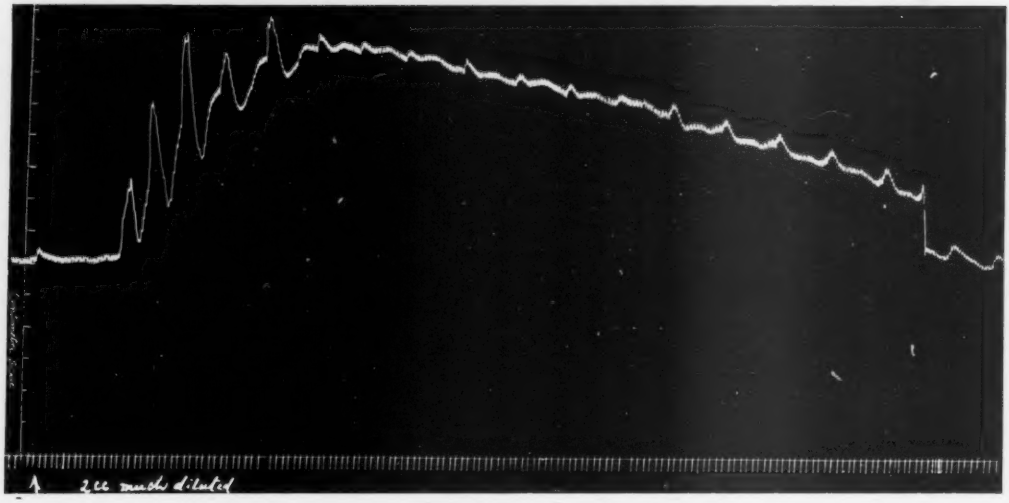


FIG. II. RABBIT'S HEART

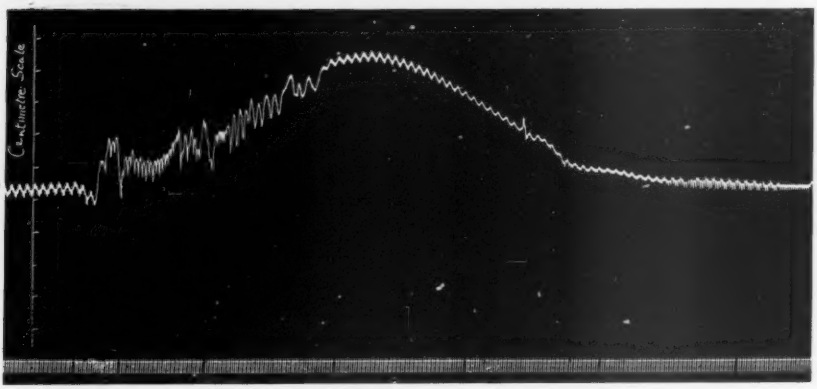
Isolated and perfused with oxygenated Ringer solution. At A the heart receives Ringer solution, to which a couple of c.c. of dilute alkaloid hydrochloride has been added.



↑A

FIG. III. DOG. VAGI CUT

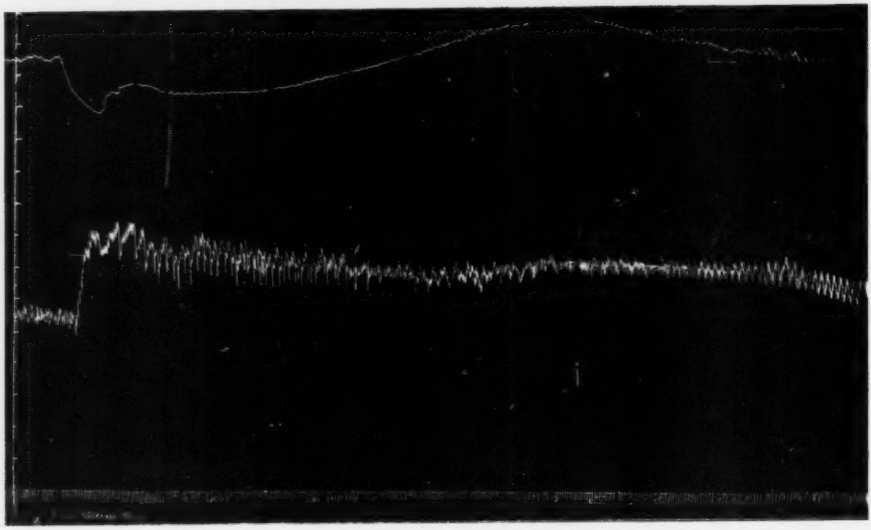
At A, 2 c.c. dilute alkaloid hydrochloride injected into jugular vein. Convulsions followed.



↑A

FIG. IV. SMALL DOG UNDER CURARE

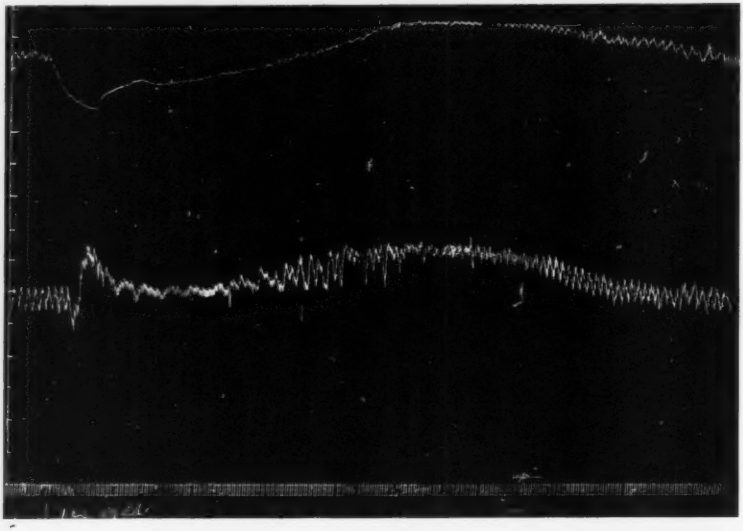
Artificial respiration. At A, 2 c.c. dilute alkaloid hydrochloride injected into jugular vein.



↑ A

FIG. V. DOG. CURARE AND ARTIFICIAL RESPIRATION.

At A, 1 c.c. of steam distillate, *i.e.* containing free alkaloid injected into jugular vein. Note rise of B.P. and slowing of heart, accompanied by fall of plethysomograph lever. Rapid heart follows, and passive rise of plethysmograph.



↑ A

FIG. VI. DOG. CURARE.

At A, 1 c.c. steam distillate into jugular vein. The lever recording Intestinal Volume was slightly ahead of that recording B.P.

CHART PRESENTATION ON RECENT WORK ON INDICATORS

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During recent years the change of mental attitude of scientific workers towards indicators has been very pronounced. The advent of the hydrogen electrode, and the results obtained by its use in the hands of Friedenthal[1] and Salm,[2] have resulted in the addition of data founded on a quantitative basis to the empirical rules of ten years ago. In a recent paper, S. P. L. Sörensen[3] has published the results of an investigation of the hydrogen ion concentration of various mixtures of pure substances and the corresponding colour changes of an exhaustive list of indicators.

'Reaction' and 'Hydrogen ion Concentration'

It will be necessary in the ensuing description to affix, for the time being at any rate, explicit meanings to some terms frequently employed. By means of hydrogen electrodes it is possible to determine directly the hydrogen ion concentration of a solution (expressed, of course, in terms of the normal hydrogen solution, one gram per litre). The value for any solution is conveniently expressed as a power of ten (that is to say, the common logarithm), and indicated by the symbol p_H^+, the negative sign being understood. For instance, centinormal hydrochloric acid is 0·00916 N with respect to its hydrogen ions, and the hydrogen ion

1. H. Friedenthal, *Zeitschr. f. Electrochemie*, Vol. X, p. 113, 1904.

2. Ed. Salm, *ibid.*, Vol. X, p. 341, 1904; Ed. Salm and H. Friedenthal, *ibid.*, Vol. XIII, p. 125, 1907, &c.

3. S. P. L. Sörensen, *Comptes Rendus des Travaux du Laboratoire de Carlsberg, Copenhagen*, Vol. VIII, pp. 1-168, 1909; Vol. VIII, pp. 396-401, 1909.

concentration is expressed conveniently as $10^{-2.04}$ the index $-2{\cdot}04$ being the logarithm of 0·00916, or it is said that the value of p_H^+ for centinormal acid is 2·04.

Now experiments on the inversion of saccharose by acids and other similar phenomena, the precipitation of colloids, the optimum conditions for enzyme actions, and so on, show distinctly that those properties which are common to all acid solutions, and which we usually put down to their 'acidity,' are quantitatively dependent upon the concentration of the hydrogen ions, i.e., the value of p_H^+.

With reference to indicators, it may be stated that when an indicator assumes a certain tint intermediate between those of its strongly acid and alkaline solutions, it shows, within limits of accuracy which experiment can determine, that the solution in which it is dissolved has a certain definite hydrogen ion concentration. Further, when another solution is observed to have the same tint under identical conditions of temperature and dilution, this second solution is found to have the same hydrogen ion concentration as the first.

In brief, the *absolute* hydrogen ion concentration can be measured in many ways, but most conveniently and directly by the potential set up between the solution and hydrogen: the *identity* of the hydrogen ion concentrations of two solutions can be established by the careful use of indicators.

The process of 'titration' is of quite another kind. It is the determination of the proportions in which two liquids must be mixed in order that the mixture may give, with an indicator, a certain result previously agreed upon—that is to say, in order that the mixture may have a certain value of p_H^+.

It must be fully realised that the titration values (c.c. of N/10 alkali to be added per 10 c.c. of solution, let us say, in order to obtain a mixture having value $p_H^+ = 5{\cdot}1$) of a number of solutions having, say, $p_H^+ = 1{\cdot}3$, may be very different indeed. A solution of glycocoll and hydrochloric acid having $p_H^+ = 1{\cdot}3$ will need far more caustic soda to bring the value of p_H^+ to 5·1 than will a solution of pure hydrochloric acid having the same hydrogen ion concentration.

The Chart compiled from curves and tables given in Sörensen's Paper

Sörensen's results can be conveniently displayed by means of the accompanying diagram, in which the behaviour of indicators towards

mixtures of known hydrogen ion concentration is graphically represented, and which I have constructed from the various tables and curves given in the original paper.

The following solutions were prepared: —

(1) Decinormal hydrochloric acid, designated in the drawing simply 'HCl.'
(2) Decinormal caustic soda, 'NaOH.'
(3) A decinormal solution of glycocoll containing in 1 litre 7·505 grams glycocoll and 5·85 grams sodium chloride, 'glycocoll.'
(4) 1/15 molecular primary potassium phosphate containing in 1 litre 9·078 grams, 'KH_2PO_4.'
(5) 1/15 molecular secondary sodium phosphate containing in 1 litre 11·876 grams, 'Na_2HPO_4.'
(6) 1/10 molecular secondary sodium citrate containing in 1 litre 21·008 grams hydrated citric acid and 200 c.c. of normal caustic soda, 'Na_2H Cit.'
(7) A solution containing in 1 litre 12·404 grams boric acid and 100 c.c. of normal caustic soda, 'NaH_2BO_3.'

These are from pure chemical substances, and can be re-made at any time. The values of p_H^+ were determined for these solutions and for binary mixtures of them, and the results are plotted in the middle portion of the diagram. Each curve represents one binary mixture prepared from two of the above solutions. The ordinate at any point in a curve represents the value of p_H^+ and the abscissa the number of parts, in ten of the mixture, of the 'less acid' or 'more alkaline' constituent.

For instance, in the case of the curve for a mixture of 'glycocoll' solution and deci-normal 'hydrochloric acid' solution the ordinate at A ($p_H^+ = 1·04$) represents the value of p_H^+ for 'hydrochloric acid,' and that at B ($p_H^+ = 6·11$) value for the 'glycocoll' solution (the less acid of the two constituents), and that at C($p_H^+ = 2·6$), the value for a mixture of 3 c.c. of the N/10 'hydrochloric acid' and 7 c.c. of the 'glycocoll' solution.

Conversely from the curves can at once be read what solutions and what proportions of them must be taken to obtain a mixture of any desired value of p_H^+ between 1·0 and 13·0.

The straight line represents a mixture of *absolute neutrality*, or, in other words, pure water. The product of the concentrations of the hydrogen and hydroxyl ions in aqueous solutions is, in accordance with accepted views of mass action, constant, and its value, experimentally determined, is $10^{-14·14}$. Utilising this fact, the hydroxyl ion concentration may be simply calculated from the hydrogen ion concentration, and this indirect method is the best at present available for the determination of $p_{\bar{O}H}$. Representing by $p_{\bar{O}H}$ the negative

exponent of the hydroxyl ion concentration then we may write that for aqueous solutions

$$p_{\bar{O}H} = 14{\cdot}14 - p_H^+$$

This relationship is expressed in the diagram. On the right hand side the numerical value of the negative exponent of the hydroxyl ion concentration may be read off directly.

On the left are drawn rectangular spaces each indicating the range of hydrogen ion concentration over which the indicator represented by that space changes its tint. Litmus, for example, it is seen, shows colour changes from about $p_H^+ = 4{\cdot}5$ to $8{\cdot}3$. The relationship of indicators one to another is shown at a glance, and it can be seen, at once, which indicators will be sensitive to slight changes of ionic concentration for any given value of p_H^+.

Sörensen has examined many indicators, and has divided them into five groups. The ranges of these groups are represented by the rectangles on the extreme left.

At the right of the diagram I have introduced the results of a few observers on the values of p_H^+ for some of the more familiar physiological fluids.

Certain limitations of the colorimetric method of determining p_H^+ must be recognised and corresponding precautions taken if reliable results are to be obtained. As these are dealt with under the heading 'Sources of error of the method' in Sörensen's paper, their repetition here is unnecessary.

An attempt has been made in the chart, by broken lines at the end of the frame in which the words 'congo-red' are placed, to show the peculiar nature of this indicator.

The chart was first prepared for my own use in connection with a method for titrating pigmented physiological fluids.[1] Its convenience for reference to laboratory workers who have to consider the behaviour of solutions whose reactions are nearly neutral, and the possibility of its use to teachers elucidating the theory and uses of indicators, have led me to its publication.

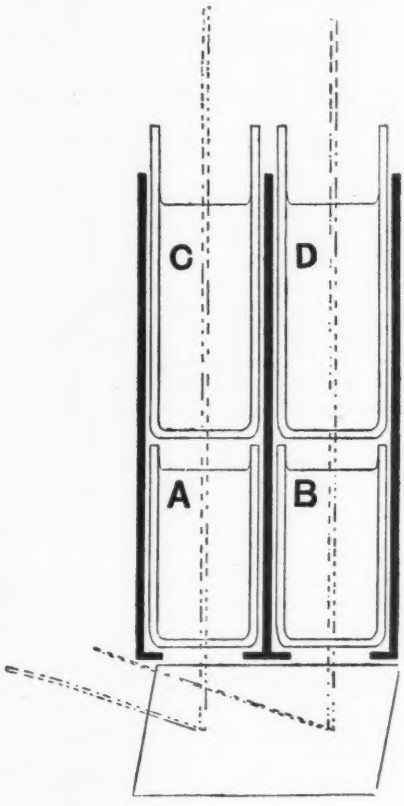
I have added a few examples illustrating the use of the chart.

EXAMPLE I.—*The determination of the hydrogen ion concentration of a given sample of urine.*

Small quantities of urine are placed in each of a series of test tubes, and trials made with a few indicators. It is found to be within the limits of the colour range of litmus and neutral

1. *Proc. Physiol. Soc.*, Vol. XL, p. 27, 1910.

red, but is just outside that of alizarin. Reference to the chart informs us that it is also within the region of colour change of rosolic acid, and, in fact, a number of the indicators of Group III of Sörensen's classification. This is the group he calls the 'Group of the phosphate indicators.' Also, that the mixtures of 'Na_2HPO_4' and 'KH_2PO_4' will serve as standard mixtures in this case. Other mixtures such as 'glycocoll' and 'NaOH,' 'sodium citrate and HCl,' will not do; the former because they are too alkaline, the latter because they are too acid. The choice of



indicator will depend upon the approximate value of p_H^+, and the colours of the indicator and the fluid under investigation, due regard being paid, also, to the 'sources of error' to which reference has already been made. In this instance litmus is chosen as the indicator, and the phosphate mixture is chosen as the standard. As the liquid is pigmented, it will be necessary to adopt either the technique of Sörensen where neutral dyes are used to compensate for the colour of the fluid under investigation or the method used by myself where a second example of the same fluid compensates for its colour.

Using the technique of Sörensen, 10 c.c. of urine are taken in a test tube, and it is found after trial with various neutral dyes that three drops of Bismark Brown solution, and one drop of Primrose Yellow solution, added to 10 c.c. of distilled water match it exactly. A number of mixtures are prepared in test tubes.

	1	2	3	4	5	6
'KH_2PO_4'	2·5 c.c.	2·0 c.c.	1·5 c.c.	1·0 c.c.	0·5 c.c.	0 c.c.
'Na_2HPO_4'	7·5 c.c.	8·0 c.c.	8·5 c.c.	9·0 c.c.	9·5 c.c.	10 c.c.

Three drops Bismark Brown, one drop Primrose Yellow, and three drops of litmus in all tubes.

To 10 c.c. of the urine in a similar tube three drops of litmus are added, and it is found that it matches Tube No. 1 above.

If, instead of using dyes, the modification of this method which I have adopted be employed the procedure is as follows.

Four glass tubes, A, B, C, D, are arranged as indicated in the diagram in a sort of box with blackened inner walls. Two diaphragms are fitted below, so that a white surface may be seen looking either down C and A or D and B. In A and B are placed 10 c.c. of urine, and in B and C equal quantities of litmus solution. In D 10 c.c. of water are placed, and to C are added the phosphate solutions from burettes in such proportions that when the volume of phosphate mixture reaches 10 c.c. the colours seen on looking down CA and DB match. It is, perhaps, more convenient to prepare some tubes, C′, C″, C‴, with slightly differing mixtures of phosphates, and to pick out the one that matches most closely. It is found, as before, that 2·5 c.c. 'KH_2PO_4' and 7·5 c.c. 'Na_2HPO_4' solutions fulfil these conditions.

As a result of either operation it is concluded that a mixture of 2·5 c.c. of 'KH_2PO_4' and 7·5 c.c. of 'Na_2HPO_4' behave to litmus as the urine does under conditions designed to determine the hydrogen ion concentration. On referring to the chart it is seen that the value of p_H^+ for this particular phosphate mixture is 7·2, and it is concluded that this is also the value for the sample of urine.

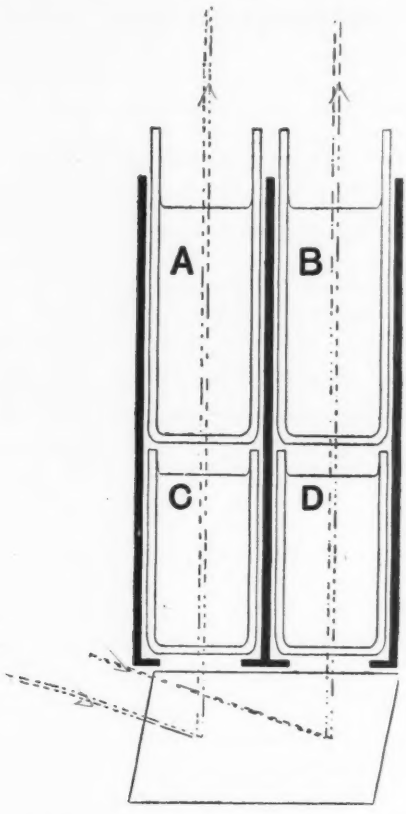
EXAMPLE II.—*The titration of a given sample of urine.*

If a titration is to be performed it is necessary to choose an 'end-point' corresponding to a certain value of p_H^+. The value naturally chosen in many cases is 7·07, that for pure water. It is convenient for use with litmus, as it falls within its range.

For the present purpose the value 9·2, that of 'Na_2HPO_4' solution, might be chosen as end-point were it not that the precipitation of calcium phosphate adds a complication to the process. Let the value 4·5, that of 'KH_2PO_4' be chosen. It is required then to discover how much decinormal hydrochloric acid solution must be added in order to decrease[1] the value of p_H^+ from its original value (we have determined it to be 7·2) to 4·5. It must not be forgotten that the tint of the pigment frequently changes (as in this particular case) with the reaction of the medium, so that in the process of *titration* the duplicate tube must match the solution *after* titration. This factor does not, however, enter into the determination of the hydrogen ion concentration.

1. In accordance with the convention with regard to the omission of the negative sign, the word *decrease* has been used here. It is obvious that the addition of acid to the solution will increase the absolute value of p_H^+, although the integral value of the exponent will be decreased, since it is a negative quantity.

Using the apparatus mentioned above, the tubes A, B, C, and D are arranged



In C are placed 10 c.c. of 'KH_2PO_4', the value of p_H^+ for which is known to be 4·5, together with three drops of litmus. In D 10 c.c. of water are placed. In A and B are 10 c.c. of urine. Three drops of litmus are also added to B, and then N/10 HCl solution is run in till an approximate correspondence is seen on looking down the two columns. It is found that 2·8 c.c. bring about this result. The like volume, 2·8 c.c., is now run into A, so that the natural pigment in A is made to have the same colour as it has in B. A slight addition to B will now result in a good match, and finally it is found that 2·9 c.c. added to both A and B give the desired result. It is, therefore, stated that, within the limits of error involved in the method, the titration value of 10 c.c. of this sample of urine is 2·9 c.c. N/10 HCl when the end-point chosen is $p_H^+ = 4{\cdot}5$.

Certain statements, some of them of an empirical nature, relating to indicators will perhaps be understood and memorised more readily after reference to the chart. Some examples are given:—

'Phenolphthalein titrates even weak acids' is a mode of expressing the fact that the pink colour of this indicator does not appear till the negative exponent of the hydrogen ions in the solution is large. Solutions having the value p_H^+ between, roughly, 7·5 and 8·0 are said to be 'acid to phenolphthalein and alkaline to litmus.' Referring to the testing of new glass for liberation of alkali in contact with water it is frequently stated that 'Rosolic acid is extremely sensitive to alkalis.' This is readily understood when it is seen that its colour change occurs when the logarithm of the hydrogen ion concentration is just numerically greater than that of distilled water. The colloidal nature of Congo-red renders its behaviour exceptional, and for the purpose of the determination of p_H^+ colorimetrically it is useless in that the results are inaccurate. Roughly speaking, however, its position in the diagram indicates that it 'reacts to strong acids, and not weak ones,' and its relations to p_H^+ values are similar to those of the indicators of Group I.

In collecting results of various observations of p_H^+, it is not to be forgotten that these are logarithms and the arithmetical mean value of the logarithms is the geometrical mean of the corresponding concentrations.

A NOTE ON CRYOSCOPY OF BLOOD

By W. R. G. ATKINS, M.A., *University Chemical Laboratory, Trinity College, Dublin.*

(Received June 22nd, 1910)

While the great degree of constancy in the value of Δ for blood of the same kind is a matter of surprise on first studying the subject, it appears that occasionally the variation may be very great. This is usually a rise in osmotic pressure, as in kidney disease, where I have found $\Delta = 0{\cdot}62°$ C., and many higher values have been recorded, the normal value in man being 0·56° C. In cases of severe haemorrhage a lowering may be produced, 0·49° C. being the lowest in my experience, though, quoting from memory, values as low as 0·47° C. have been met with.

In this connection it may be of interest to record the values obtained with two specimens of monkey's blood which I was enabled to get from the Dublin Zoological Gardens through the kindness of Prof. A. F. Dixon—

Cynocephalus babouin, blood $\Delta = 0{\cdot}615°$ C.
Rhesus ? blood $\Delta = 0{\cdot}605°$ C.

The blood was obtained from the hearts of the animals, which had died from colds on the previous day. Accordingly the values may not be quite normal.

One specimen of hare's blood was observed to give the very abnormal value $\Delta = 1{\cdot}125°$ C. This measurement was performed with the greatest care. The blood was sent to me, in a well-corked clean bottle, sent from the laboratory for the purpose, and was perfectly fresh. Hamburger[1] records $\Delta = 0{\cdot}578°$ C. for the whole blood and 0·580° C. for the serum. Winter[2] gives 0·570° C. for the serum.

Another exceptional value was given by the first in a series of samples of blood of the common fowl, $\Delta = 0{\cdot}935°$ C., while the mean value was $\Delta = 0{\cdot}607°$ C.[3] It was only after examining duck's blood, $\Delta = 0{\cdot}574°$ C., that the abnormality was suspected, and then many other specimens of fowl's blood were examined.

1. Hamburger, *Archiv. f. Anat. u. Phys.*; *Phys. Abt.*, S. 281, 1895.
2. Winter, *Archiv. de Physiol.*, 1895.
3. Atkins, *Proc. Roy. Dublin Soc.*, Vol. XII, N.S., March, 1909.

A large specimen of *Rhina squatina* also afforded a very puzzling value $\Delta = 0{\cdot}61°$ C., while the water of the bay gave $\Delta = 1{\cdot}70°$ C. This Elasmobranch should, according to the numerous observations of Bottazzi, C. W. Greene, W. E. Garrey and W. J. Dakin, have been practically isotonic with the sea-water. It was found on a mud flat in Cork Harbour, and the blood drawn from the heart immediately after death. The tide was falling at the time, and though the river is tidal for about ten miles higher up, it is possible that the *Rhina* lost most of its blood salts in the fresher water higher up, and was then carried down in a feeble condition and stranded.

Below are recorded a few results obtained with the blood and ovarian eggs of fishes. These do nothing more than confirm the results of W. J. Dakin,[1] and the experiments were discontinued when it was seen that the question had already been fully investigated. I am indebted to Mr. S. W. Kemp, of the Irish Fisheries Department, for most of the specimens. It was noticed that the blood was unclotted in the majority of cases, even after keeping a week in a well-corked bottle. Delezenne had previously noticed this phenomenon, and Rodier[2] records that coagulation is hastened by buccal mucus and peritoneal fluid to a greater degree than by tissues of the fish.

Fish	Fluid	Δ	Remarks
Salmo	Ovarian fluid	0·56	From fresh water
Raia circularis	Blood	2·095	Not clotted, S.R. 745
Centrophosus squamosus	Ovarian egg	2·070	Pale yellow, viscous
,, ,,	,,	2·098	,, ,,
,, ,,	Blood	2·125	Not clotted, S.R. 745

Station	Depth	Δ
Atlantic, off Irish coast, S.R. 745 ...	Surface ...	1·978
,, ,, ,, ...	100 fathoms ...	1·976
,, ,, ,, ...	250 fathoms ...	1·979

Fish from W. 67	Fluid	Δ	Remarks
Gadus morrhua	Blood	0·827	From 3 specimens, clotted
Gadus virens	Blood	0·858	From 1 specimen, clotted
Molva molva	Blood	0·747	From 1 specimen, not clotted
Mustelus vulgaris	Blood	2·160	From 1 specimen, not clotted
Acanthias vulgaris	Blood	2.100	From 7 specimens, not clotted
,, ,, ...	Ovarian egg	2·047	Pale yellow, viscous

Sea Water

Station	Depth	Δ
W. 67	Surface ...	1·950
,, ...	20 fathoms ...	1·950

1. Dakin, *Bio-Chemical Journal*, June and December, 1908.
2. Rodier, *Stat. Zool. d'Arcachon Univ. Bordeaux, Trav. des Lab.*, pp. 129-132, 1900-1901.

URIC ACID METABOLISM IN DOGS *

By H. ACKROYD, M.A., M.D., *British Medical Association Research Scholar.*

From the Pharmacological Laboratory, Cambridge

(Received June 29th, 1910)

Wiechowski[1] in 1907 showed that preparations of the livers of dogs and oxen when incubated with sodium urate for about eight hours converted the uric acid into allantoin. In each of his three experiments the uric acid disappeared completely, urea could not be isolated, and allantoin was obtained in the pure crystalline state. Only the second of these was quantitative, and in this 75 per cent. of the uric acid which had been destroyed was converted into allantoin. From this he concluded that uric acid is converted quantitatively into allantoin under the conditions of his experiments while admitting that more prolonged incubation, or incubation under anaerobic conditions, might possibly lead to different results (p. 305). It may also be added that the mode of preparation of his ferment solutions might favour the production of allantoin and not that of other substances; moreover, 25 per cent. of the uric acid which had been destroyed was not accounted for in his experiment. He made an exhaustive criticism of the work of previous observers, and showed clearly that no satisfactory proof had been brought forward that urea, whether by way of glycocoll or allantoin, was the nitrogenous end point of purin metabolism in mammals.

In a later paper[2] he describes a new method of estimating allantoin, and by the use of this showed that it was a constant constituent of normal urine of dogs, rabbits, cats and a monkey. Although allantoin had been found in the normal urine of calves,[3] dogs, cats, rabbits[4] and a cow,[5] it had not been regarded as constantly present in the urine of the lower animals, but rather as an occasional occurrence or produced by exceptional circumstances, such as poisoning by hydrazin,[6-8] limitation of air supply,[9] or administration of excessive quantities of purin substances.[10-19] Wiechowski put forward the view that in the lower animals allantoin is the normal end product of purin metabolism, and

* The expenses of this work have been defrayed by a grant from the Royal Society.

that none of the urea excreted is derived from uric acid. He supported this opinion by metabolism experiments on dogs and rabbits. In one experiment uric acid injected subcutaneously into dogs was excreted to the extent of 59 per cent. as allantoin (the uric acid excreted was not estimated in this experiment); while in another (p. 116) 21·4 per cent. of the uric acid injected was excreted unchanged and the whole of the remainder appeared as allantoin. In this last experiment the excretion of urea was much increased after the injection of uric acid, and it cannot be regarded as clearly shown that some of the allantoin excreted might not have been due to the effect of a large dose of uric acid on the endogenous purin metabolism, while a part of that actually injected might have been excreted in some other form; at any rate, the increased excretion of urea was out of all proportion to the nitrogen of the uric acid administered. Uric acid given to a rabbit by the mouth was excreted to the extent of 92 per cent. as allantoin, while when injected subcutaneously into another rabbit 5·6 per cent. was excreted unchanged and 53·2 per cent. as allantoin. In this case the whole nitrogenous metabolism was altered by the injection, and Wiechowski considered that the subcutaneous method should not be employed in rabbits owing to this effect on metabolism. The same objection to subcutaneous or intravenous injection for metabolism experiments applies most probably to other animals (Schittenhelm and Seisser[20]); the method of administration is abnormal, the substance given not reaching the liver at once, but acting first, if it have any action, on other tissues.

In the case of dogs, Schittenhelm[21] has brought forward strong support in favour of Wiechowski's view; he administered thymo-nucleic acid by the mouth, and recovered in three experiments 102 per cent., 99 per cent. and 88 per cent. of the purin nitrogen given as purin bases, uric acid, or allantoin; of this, 95 per cent., 94 per cent., and 93 per cent., respectively, was excreted as allantoin. In another experiment, in which the animal was starving, 98 per cent. of the purin substance given was recovered, and of this 95 per cent. was allantoin. Schittenhelm, however, gives an observation (p. 83) concerning Wiechowski's method of estimating allantoin. According to this, the allantoin appears to be greater in quantity when calculated from the nitrogen of the mercury-acetate precipitate than when isolated from that precipitate in the pure state. He found that this difference might amount to as much as 30 per cent., which seems to indicate that nitrogen containing substances other than allantoin may be carried down by mercury acetate. Since

Schittenhelm seems to have calculated his results from the nitrogen, his values for the allantoin excretion may be too high; they certainly show that allantoin is the principal form in which purin substances are excreted by dogs, but there remains the possibility that other substances, perhaps urea, might also be formed, though in small amounts. This would, perhaps, have little practical interest were it not that Wiechowski[22] has recently revived the older views of Loewi,[23] and of Soetbeer and Ibrahim,[24] that man has not the power to destroy uric acid but excretes it all unchanged. At first he was unable to isolate allantoin from human urine, but later[25] he showed that man regularly excretes about ten milligrams of allantoin daily: this observation was confirmed by Schittenhelm and Wiener.[26] Wiechowski takes this quantity (0·01 g. per diem) as the measure of man's power to destroy uric acid, but Schittenhelm and Franz[27] consider that man readily destroys uric acid and converts it into urea, or more correctly into some substance, not allantoin, which is estimated as urea by the Pflüger-Bleibtreu method.

It seems quite possible that uric acid metabolism in the lower animals may proceed in two directions, the one leading to allantoin and perhaps ending there, the other to urea or intermediary bodies. Nor is it improbable that these different mechanisms may be used to a varying extent by the same animal under different metabolic conditions, and still more is this likely to be the case in different classes of animals. The lower animals may possess both mechanisms but use chiefly that producing allantoin, while in man little allantoin may be formed, uric acid metabolism proceeding mainly in another direction. It was from this standpoint that the experiments on dogs here recorded were undertaken with the view of ascertaining whether these animals had the power to convert uric acid into any other substance than allantoin.

The method of perfusion of the surviving liver was chosen for the investigation, since thereby the normal balance of ferment action is less disturbed than by the use of organ extracts, whether partially purified or not. The perfusions were carried out in a modified form of the apparatus described by Brodie and Dixon.[28] No food was given to the dogs for twenty hours before the experiment, they were killed by pithing, cannulae were tied into the portal vein and superior vena cava, the liver was washed free from blood with normal saline solution* followed by fluid of the same

* Normal saline solution as used in these experiments was of the following composition :—Sodium chloride, 0·9 g. per cent.; potassium chloride, 0·025 g. per cent.; sodium bicarbonate, 0·015 g. per cent.; calcium chloride, 0·02 g. per cent, and distilled water Ao 100 per cent.

composition as that subsequently used for perfusion, thus preventing any dilution of the perfusion fluid.

The liver was then excised and placed in the perfusion chamber. Nine hundred cubic centimetres of the perfusion fluid, kept well oxygenated, were passed through the liver in a continuous stream, the whole quantity passing through about ten times per hour by force of gravity alone (the fluid ran down from a container placed 50 cms. above the portal vein). The temperature both of the fluid and of the chamber containing the liver was kept at 38° to 40° C.

At the end of the experiment, proteid was removed from the perfusion fluid by acidifying with acetic acid and boiling, the fluid when cool was made up to its original bulk with distilled water and the coagulated proteid filtered off. Uric acid was estimated by the Ludwig-Salkowski method. Attempts were made to isolate urea by the method given in Hoppe-Seyler's Hdb., 8th edit., p. 651. Allantoin was estimated by Wiechowski's method, the very dilute solutions being previously concentrated on the water bath to a known volume so as to avoid great dilution in the final precipitation; the quantity present was afterwards calculated in terms of the original solution, of which never less than 600 c.c. were used. The allantoin was always isolated and weighed. Allantoin was identified by its crystalline form, its melting point with decomposition at 231° C., and when sufficient material was obtained, by its percentage nitrogen composition determined by Kjeldahl's method (theoretical = 35·44 per cent.). Its solutions tested with silver nitrate alone gave no precipitate, but a voluminous one on further adding a little ammonium hydrate; this was soluble in excess, but came down again on adding more silver nitrate. A trace of the solid substance boiled with strong sodium hydrate gave the glyoxylic reaction, while addition of acetic acid and calcium chloride brought down microscopic crystals of calcium oxalate.

Experiment I.—Normal saline solution was perfused for one hour. No urea could be isolated from the perfusion fluid at the end of the experiment.

Experiment II.—Normal saline solution containing 0·076 g. per cent. of uric acid as sodium urate was perfused for two and a half hours. At the end of the experiment the percentage of uric acid was 0·0504 g. No urea could be isolated from 140 c.c. of the solution.

Experiment III.—Normal saline solution containing 0·1554 g. per cent. of uric acid dissolved by the addition of a minimal quantity of sodium hydrate was perfused for two hours. At the end of the experiment the percentage of uric acid had fallen to 0·0947 g. No urea could be isolated by Hoppe-Seyler's method from 272 c.c. of the solution, but 0·0625 g. of a nearly white substance crystallising in fine prisms like allantoin was obtained. When heated this

began to brown at 220° C., and melted with decomposition at 225° C. It gave the reactions described for allantoin, and was precipitated from solutions by Wiechowski's mercury acetate reagent, not by phospho-tungstic acid or basic lead acetate.

Experiment IV.—Normal saline solution containing 0·0645 g. per cent. uric acid as sodium urate was perfused for two hours. The uric acid at the end of the experiment had fallen to 0·045 g. per cent. No urea could be isolated from 280 c.c. of the fluid, but a few crystals microscopically resembling allantoin were obtained; the quantity was not sufficient for further investigation.

Experiment V.—Normal saline solution containing 0·1048 g. per cent. of uric acid brought into solution by a minimum quantity of sodium carbonate was perfused for one and a half hours. At the end of the experiment the percentage of uric acid had fallen to 0·092 g. per cent. No urea could be isolated from 350 c.c. of the fluid.

These experiments seem to indicate that the liver of dogs, while able to destroy uric acid, cannot convert it into urea, but on the other hand allantoin is formed. Further experiments were carried out to ascertain if the allantoin produced represents quantitatively the uric acid destroyed.

Experiment VI.—A dog's liver was perfused for five hours with normal saline solution. At the end of the experiment no uric acid was present in the solution, but by Wiechowski's method allantoin was found to be present to the extent of 0·011 g. per cent. This allantoin, when heated, began to brown at 225° C., and melted with decomposition at 229° C. It burnt completely on platinum foil, leaving no residue; it crystallised in fine prisms, and its chemical reactions were those already given for allantoin.

Experiment VII.—A dog's liver was perfused for five hours with normal saline solution. At the end of the experiment no uric acid was present, but the fluid contained 0·0079 g. per cent. of allantoin, melting with decomposition at 228° C., having the typical crystalline form, and giving the chemical reactions of allantoin.

Experiment VIII.—A dog's liver was perfused for five hours with normal saline solution containing 0·0542 g. per cent. of uric acid as sodium urate. At the end of the experiment the percentage of uric acid was 0·024 g. From 820 c.c. of the fluid 0·1585 g. of allantoin were isolated. This melted at 230° C., contained 36 per cent. of nitrogen (theoretical for allantoin is 35·44 per cent.), and in crystalline form and chemical reactions agreed with allantoin.

Experiment IX.—Normal saline solution containing 0·0758 g. per cent. of uric acid a sodium urate was perfused for five hours. At the end of the experiment the fluid contained 0·0422 g. per cent. of uric acid and 0·019 g. per cent. of allantoin, which melted at 228° C., burnt, leaving no residue, crystallised in prisms, and gave the usual chemical reactions.

Experiment X.—Normal saline containing 0·0447 g. per cent. of uric acid as sodium urate was perfused for five hours. At the end of the experiment the percentage of uric acid was 0·0181 g. and the fluid contained 0·0243 g. per cent. of allantoin, which melted at 226°C., contained 35·44 per cent. of nitrogen, crystallised in typical prisms, and gave the usual chemical reactions.

The control experiments VI and VII show that in five hours' perfusion of a dog's liver with normal saline solution a quantity of

allantoin, approximately 10 mgs. per cent., is produced from the liver itself, while any uric acid formed is at once decomposed. Whether this allantoin is to be regarded as part of the normal endogenous metabolism of the liver, or as the result of early death of the tissues, cannot be certainly decided, but the absence of uric acid and the close agreement of the two experiments, quantitatively, seems to be in favour of the former view. In none of the other experiments VIII, IX and X was the uric acid which had been destroyed replaced even approximately quantitatively by allantoin. Allowing 10 mgs. per cent. as the amount of allantoin produced from the liver in a five hours' perfusion, the percentage of uric acid destroyed which was recovered as allantoin in these three experiments was 32·7, 28·5 and 57·2. It is possible that more allantoin had been formed but had been converted into some other substance. The following experiment shows that this was not the case.

Experiment XI.—Normal saline solution containing 0·2576 g. per cent. of allantoin (Merck) was perfused for five hours. At the end of the experiment the solution contained 0·2571 g. per cent. of allantoin having a melting point of 231° C., and containing 35·52 per cent. of nitrogen.

Allowing for 10 mgs. per cent. produced during the perfusion, this gives 96 per cent. of the allantoin recovered in the pure state, which agrees with the observation of Poduschka,[7] who found that of allantoin administered to dogs 90 per cent. could be recovered unchanged in the urine, while Minkowski,[13] using the same animals, recovered 70 per cent.

In all the last six experiments an attempt was made to isolate urea from the filtrate after precipitation with mercury acetate by addition of mercuric nitrate; none, however, was found.

These experiments show that the liver of dogs can destroy uric acid and can partially convert it into allantoin, but the amount of this substance formed falls far short of corresponding in quantity to the uric acid destroyed, when allowance is made for the amount of allantoin produced from the liver itself during perfusion. The deficit could not be accounted for as urea, nor could Ascoli,[29] in his perfusion experiments (experiment XIII, p. 344), isolate urea in the pure crystalline state, though he believed that it had been produced. This opinion rested on estimations by the Pflüger-Schöndorf method, which would, however, include allantoin as urea. The same objection applies to Subkow's[30] perfusion experiments, the urea being estimated by a nitro-metric method in the filtrate from precipitation by phospho-tungstic acid. Although perhaps too much weight should not be put on the quantitative side of

perfusion experiments, I think it is extremely unlikely that the uric acid unaccounted for as allantoin had been retained in the liver either mechanically or physiologically, and the amount of the deficit is far too great to be accounted for by experimental error. It seems, therefore, more likely that allantoin is not the sole end product of purin metabolism in dogs, though it is undoubtedly the main one. Since dog's liver cannot destroy allantoin, any other substances formed from uric acid must originate in a primary cleavage of the molecule other than that by which allantoin is formed. Glycocoll was believed by Wiener to represent an intermediate stage in the conversion of uric acid to urea in rabbits[31] and oxen,[32] while such a very simple chemical reagent as hydrogen peroxide can convert uric acid through tetra-carbonimide[33] and carbonyl di-urea to urea[34] itself. Failure in these experiments to isolate urea does not prove that it is not formed; for the amount of it or any one intermediary substance might be so small as to escape detection.

Experiments on rabbits on similar lines to these on dogs are now being carried out so as to ascertain if in those animals any decomposition products of uric acid other than allantoin can be isolated.

Summary

The liver of dogs when perfused with normal saline solution produces a small quantity of allantoin.

When sodium urate is added to the perfusion fluid, a part of the sodium urate is destroyed: part of this can be recovered as allantoin, but not the whole. No urea could be isolated from such a perfusion fluid.

The liver of a dog has not the power to destroy allantoin.

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SOME OBSERVATIONS ON THE NATURE OF THE 'BENCE-JONES' PROTEIN

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The peculiar protein found in the urine in cases of multiple myeloma has, since the time when Bence-Jones first described it,[1] been found on but few occasions, owing to the rarity of the disease with which it is associated. Notwithstanding this, a great deal of work has been done towards elucidating its characters. The result has been that the various workers have placed it in different classes, and little harmony can be found in the results obtained. This contribution, based upon the study of the protein obtained from an undoubted case, tends to show that the protein bodies found in the urine differ from time to time even in the same case, and probably the true explanation of the varying results found by different observers lies in the fact that, in the disintegration of the bones and tendons, chondro-mucins are liberated which are more or less broken up, and thus excreted differently according to the stage of the disease.

The case from which the protein was obtained was that of a miner, aged 30 years, who was first seen on January 11th of this year, when a small protrusion on the right fifth rib was found. He then complained of great weakness and a very poor appetite. His doctor, who had known the man for some time, found that he had wasted very considerably. He gradually became weaker, and on February 8th there was some rigidity in the upper dorsal spinal region. An examination of the nervous system pointed to some involvement of the spinal cord in this region, and on pressing between the spinous processes of the last cervical and first

1. *Philosoph. Trans. of the Royal Soc.*, 1848.

dorsal, and between the latter and second dorsal, the spinous ligaments were felt to be much less resistant than in any other part of the spinal column. Later, there was marked rigidity of the neck muscles, the head being thrown backwards and held stiff.

On February 12th, on examining the ribs, the first on the right side and the second and third on the left side fractured. On February 18th the sternum fractured spontaneously just below the manubrio-gladiolar junction, giving rise to marked deformity in the chest.

On the 19th the left sterno-clavicular joint became disorganised, and later the inner end of the clavicle was drawn up into the neck by the sterno-mastoid muscle. Before death, on the fifth of March, the whole of the ribs were soft and easily gave way under pressure.

A piece of rib was allowed for examination, and Dr. E. E. Glynn examined it and found that it was characteristic of the disease multiple myeloma, and analogous in all respects with the specimens prepared from a case described by Sir James Barr.

The Urine

On February 11th the urine gave the specific reactions of the Bence-Jones protein.

On warming after the addition of acetic acid, there developed at 52° C. a milky, then heavy, precipitate which disappeared almost completely at 95° C. The acetic acid gave no precipitate in the cold.

On adding nitric acid a heavy precipitate formed which was soluble on warming, and reappeared on cooling. This could be repeated several times before the protein failed to disappear on warming.

On repeated saturation with ammonium sulphate, and washing with the same fluid, the precipitate obtained, dissolved in either dilute sodium chloride or water, gave the biuret test.

It gave the test for loosely combined sulphur, and the potassium ferrocyanide and acetic acid test.

The hydrochloric acid test (Bradshaw's test) was negative.

The dialysed urine gave the same reactions.

For the first few days the urine was passed quite clear, but later it became turbid and then milky.

The nature of this protein has given rise to a great deal of controversy. Bradshaw, as the result of examination of the urine of ten

cases, states that it is an albumose. Magnus Levy, who isolated the protein and obtained it in a crystalline form, states that it is nearly related to a genuine albumin. Cohnheim considers it a coagulated albumin. Lindeman concludes that while it cannot without objections be put in any group of proteins, it is nearest the true albumins.

In the different cases recorded, the properties of the substance found have differed so much that either they were different bodies or were not tested pure.[1] That the proteins found can differ even from day to day is shown by the fact that the second specimen examined from the case under consideration (on February 13th) gave a well marked precipitate with cold acetic acid. A specimen of this precipitate was obtained from 500 c.c. of urine, purified and found to contain a carbohydrate radicle and no phosphorus; it therefore is shown to be a mucin.

The reactions varied slightly from time to time, though on most days the nitric acid test, coagulation temperature, loose sulphur, and 'salting out,' were quite typical.

On February 19th a dialysed specimen gave the hydrochloric acid reaction quite typically, and no precipitate with acetic acid in the cold.

It was clear from the outset, after finding different reactions on two successive days, that there was probably a mixture of proteins in the urine, or that a common body was being split up differently from day to day.

The dialysed specimen which gave the hydrochloric acid reaction was kept in an open vessel for two days, after which the reaction was much less marked. It then gave a precipitate with cold acetic acid (2%) which it had failed to do before. This precipitate was phosphorus free, and contained a carbohydrate radicle. The probability was, therefore, that in this case there was a conjugated protein present which under certain conditions resolved into other bodies, one of which was a mucin.

Mucin and allied compounds belong to the group of glyco-proteins. Such proteins occur in the tissues undergoing degeneration in such cases, viz., cartilage, tendon, and bone, conjugated with chondroitin sulphuric acid.

In the table there are the results of two days' complete analyses of the urine, which were performed with a view of obtaining some indications on which to work. It will be observed that there was an increased excretion of calcium, and diminished chloride excretion.

1. Emerson. *Clinical Diagnosis.*

CASE OF MULTIPLE MYELOMA

Urine	February 17th, 1800 c.c.	February 18th, 2100 c.c.
Total Nitrogen	13·5 grams	15·96 grams
Urea	27·0 „	29·61 „
Uric acid...	2·07 „	0·504 „
Ammonia (as NH_3)	0·252 „	0·336 „
Phosphates (as P_2O_5)	2·16 „	2·31 „
Chlorides (as Cl)	1·728 „	1·55 „
Calcium	0·594 „	0·714 „
Sulphates (as $BaSO_4$)—		
Organic	0·025 per cent.	0·002 per cent.
Inorganic	0·28 „	0·28 „
Ratio	1 : 11 „	1 : 160 „

Creatinin (by Jaffe's Test) was present.

	Feb. 17	Feb. 18	Feb. 22	Feb. 28	Mar. 3
Sulphate ratio (ethereal to inorganic)	$\frac{1}{11}$	$\frac{1}{160}$	$\frac{1}{10}$	Ethereal practically nil	$\frac{1}{4}$

The most characteristic feature observed was the marked change in ethereal sulphate excretion, it being normal on the first occasion and markedly diminished on the second. This observation, coupled with the fact that the Bence-Jones body contains loosely combined sulphur, suggested that the body might have some relation to the proteins which have recently been isolated from bone containing a high sulphur content.

Loebisch, Gies,[1] and others have isolated a substance from tendons which does not differ from true mucins in its properties, and Gies has prepared an identical substance from bones which he calls ' osseo-mucoid.' It also possesses a high sulphur content and contains likewise a paired sulphuric acid, thus resembling a chondro-mucoid.

It is obvious from the history that in this case both tendon and bone were being rapidly broken down, and the probability of their derivatives being found in the urine is not unlikely.

The marked variation in the excretion of ethereal sulphates with a protein in the urine containing loosely combined sulphur led us to study the relationship between these two conditions, but unfortunately before this could be completed the patient died.

1. Mann. *Chemistry of the Proteids*, 1906, p. 541.

On February 18th, from 500 c.c. of urine 0·4 grams of the protein were obtained in a pure condition. It contained 10·5 per cent. of sulphur.

On March 2nd, 0·3 grams of protein were obtained, and it contained only 0·55 per cent. of sulphur.

The variability in the nature of the protein found is thus obvious.

It is of striking interest to observe that with this change in the nature of the protein there was a variation in the output of ethereal sulphate, on the first occasion there being practically no ethereal sulphates in the urine, and on the second occasion there being an excess.

In view of the fact that the case terminated before these observations could be completed, it would be unwise to draw any fixed conclusions from these observations, but it is of extreme interest to find that in a disease where the bones and tendons containing chondro-mucoid are being rapidly broken down, proteins of a similar nature—in that they contain a carbohydrate radicle and a high sulphur content—are found in the urine.

Two other cases of bone disease which are being investigated reveal the presence of the mucin-like body, but they do not show any of the other reactions.

Another unusual feature of the case was the constant occurrence of a crystalline deposit in the urine which was found to be sodium urate, a point of interest in view of the marked increase in calcium katabolism. Professor Benjamin Moore, in the last number of this Journal, calls attention to the importance of altered calcium metabolism in gout, and it is thus a striking feature that in a case where there is obviously a marked increase of calcium katabolism, a crystalline form of sodium urate is excreted. So far, I have been unable to find any reference to a similar crystalline sodium urate in urine.

My thanks are due to Professor Benjamin Moore for his valuable suggestions as to the method of approaching the problem and his constant help; also to Dr. T. R. Bradshaw for the interest which he took in the case, and information concerning his previous investigations of 'Bence-Jones' protein.

APPARATUS FOR COLLECTING AND MEASURING THE GASES EVOLVED DURING FERMENTATION

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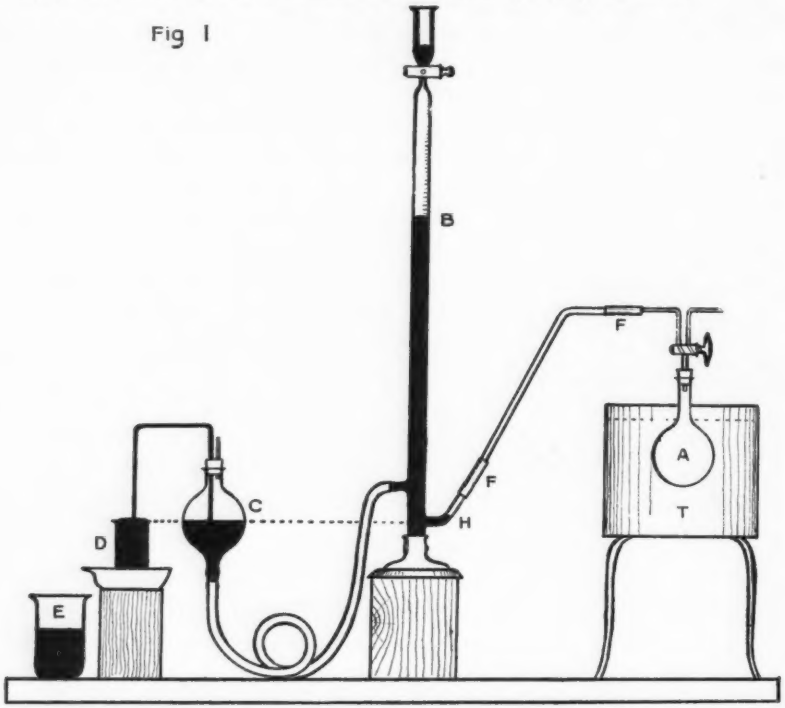
In measuring the total quantity and rate of evolution of gases evolved during fermentation, it is essential to keep the pressure in the fermentation flask as nearly constant as possible throughout the experiment. Two forms of apparatus have been in use in this laboratory for some time for this purpose, and have been found to be very simple and convenient.

1. The first form is intended for the measurement of comparatively small volumes of gas, such as those evolved in the fermentation of sugars by yeast juice,[1] and is illustrated in the accompanying figure 1.

Fig 1



1. Harden and Young. *Roy. Soc. Proc. B.*, 1906, 77, 406.

The fermentation flask (A) of 100-200 c.c. capacity, placed in the thermostat (T), is fitted with a three-way tap connected by glass tube and rubber with a Schiff's azotometer of 100 c.c. capacity, graduated to 0·2 c.c. It is essential that the rubber connections (F, F) should be as short as possible, owing to the high permeability of rubber for carbon dioxide, but at the same time it is necessary to allow so much play that the flask can be removed from the bath and shaken by hand, or mechanically agitated in the bath. The reservoir (C) of the azotometer is connected by a syphon, passing through a doubly-bored stopper, with a small cylinder or beaker (D) which stands in a flat dish of glass or porcelain provided with a spout from which mercury can flow into a collecting vessel (E). The effect of this arrangement is that the mercury displaced from B passes through the syphon into D and overflows into the porcelain dish and so into E. The mercury at C and H, therefore, remains at the level of the top of D, and this is adjusted once for all, so that the mercury just extends to the bend of the tube H, and consequently the pressure in the flask is maintained at that of the atmosphere.

The volume of gas is read on B at the reduced pressure corresponding to the height of the column BH, and is corrected by means of a table. (See below.)

When the azotometer is full of gas, the tap on A is closed, the syphon removed, the reservoir filled with the mercury from E, and the gas displaced by raising the reservoir. The syphon is then replaced, and started by means of a pressure bulb attached to a short tube passing through the stopper of the reservoir. The tap on A is then re-opened, and the collection of gas resumed as before. If required, a sample of the gas can be taken for analysis by means of the mercury cup at the top of the azotometer.

The table of corrections is constructed for an atmospheric pressure of 760 mm. by measuring the height of each cubic centimetre graduation above H, subtracting this from 760, and then calculating the corrected volume corresponding with each graduation. The corrected numbers for the intermediate graduations are found by interpolation. A table of this kind is sufficiently accurate for most purposes; when greater accuracy is required the readings must be specially corrected for the prevailing atmospheric pressure.

The rate of fermentation of a sugar by yeast-juice is determined by

the aid of this apparatus in the following matter. The azotometer is filled with mercury and the syphon adjusted in C. The desired mixture of yeast-juice, sugar and toluene is placed in A, brought to the temperature of the thermostat and saturated with carbon dioxide by removing the flask, filling it with carbon dioxide from a Kipp, replacing the stopper carrying the tap and shaking, and repeating this process until saturation is effected. If desired, the flask can be fitted with inlet and outlet tubes and the carbon dioxide blown through without removing the flask from the bath, but this is not so convenient in the case of yeast-juice as the method described above.

Since yeast-juice readily becomes supersaturated with carbon dioxide, the flask is vigorously shaken for about half a minute before each reading. The flask is then replaced in the thermostat, allowed to stand for one minute to re-establish temperature equilibrium, and the volume of gas in the azotometer read. At the expiration of the desired interval from the time of first shaking, the process is exactly repeated and the volume of gas evolved during the interval thus ascertained.

So strong is the tendency to supersaturation in the case of yeast-juice that scarcely any gas is evolved during 5-10 minutes, the whole volume produced during this time being retained in the liquid and only evolved when this is vigorously agitated.

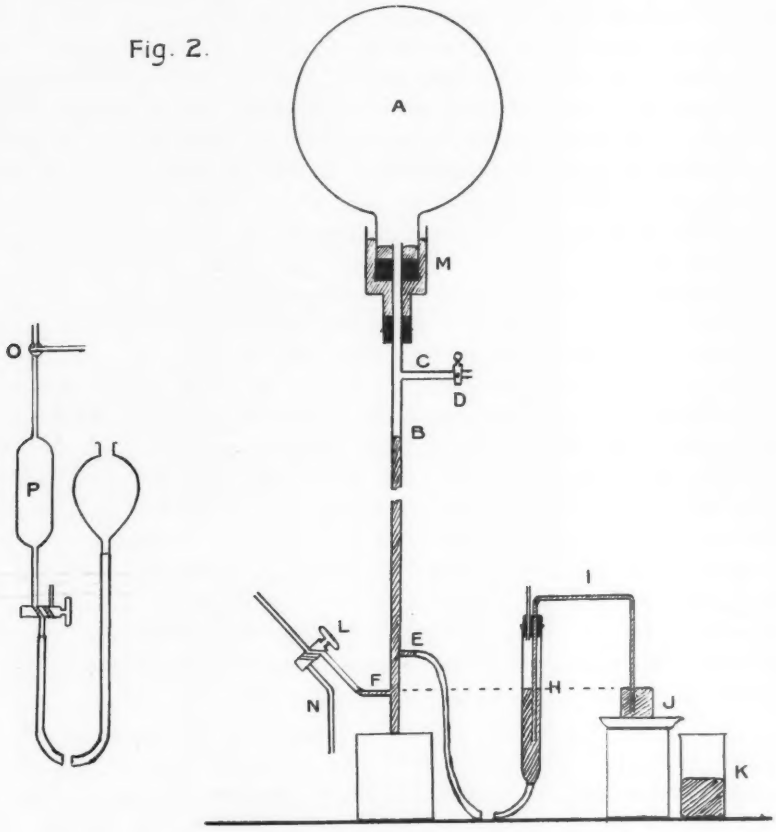
This method works extremely well so long as the volume of gas evolved does not exceed about 30 c.c. per 5 minutes, and does not fall below about 0·5 c.c.

2. The second form of apparatus is intended for the collection and measurement of the comparatively large volumes of gas which are evolved by the action of bacteria on quantities of about 20 grams of sugar, the object being the collection and measurement over mercury of a large volume of gas (3-6 litres) without employing an unmanageable amount of mercury. The apparatus is shown in fig. 2.

A large inverted bolthead (A) (3-7 litres in capacity) is fitted with a rubber stopper carrying a vertical tube (B) about 1 metre in length and 10 mm. in external diameter. This tube projects about 2 cm. inside the stopper of the flask, and carries near the top a side piece (C) fitted with a good mercury sealed glass tap (D). B is sealed at the lower end, near which are two side tubes arranged as in Schiff's azotometer (E, F). At E is attached a rubber tube and a cylindrical mercury reservoir (H) about

20 mm. in diameter and 180-200 mm. in length, and this is fitted with a syphon and overflow cylinder (I, J K) exactly like those employed for apparatus 1. The side tube (F) carries a three-way tap (L).

Fig. 2.



In order to prevent leakage, the rubber stopper of the flask A is immersed in mercury contained in a cup (M) carried on the vertical tube B by means of a rubber stopper. In order to prepare the apparatus for use, mercury is poured into the reservoir (H), and the lower part of the tube B and side tube F up to the tap are filled with mercury, which is also allowed to flow out of the upper end of B and fill the space immediately above the stopper of the bolthead. This prevents the gas from coming into

contact with rubber after it has once passed into the apparatus. The side tube C is then connected with a Fleuss pump or a mercury pump and the whole apparatus exhausted. Mercury then rises in B to the barometer height above that in H. As soon as the exhaustion is as complete as possible, D is closed; H is fitted with its syphon, and J is adjusted so that the level of the mercury is at the same height as the point at which F enters B. The height of the column BF subtracted from the height of the barometer gives the residual pressure, if any, in the apparatus, and is measured by a vertical millimetre scale. The fermentation flask, placed in an appropriate incubator or thermostat, is connected by rubber to the tube carrying the tap L, and the air of the space above the liquid in it is swept out, if this is desired, by a suitable gas through the limb N. The tap L is then turned so as to connect the flask with B, and the collection of the gas then proceeds regularly until the experiment is interrupted. Any mercury carried up by the bubbles of gas strikes against the concave surface of the bolthead and falls back again into the tube. The diameter of the side tube F must be decidedly less than that of B, or the bubbles of gas entering B drive too much mercury before them and cause violent oscillation of the column. At the close of the experiment the pressure and temperature of the gas are read off and its corrected dry volume ascertained by calculation from the known capacity of the apparatus. The calibration of the large flask is effected by means of water, and that of the tube B by means of mercury. It is necessary to know the volume of the bolthead as arranged for the experiment, and of the side tube C and tube B down to a fixed point, and the capacity per centimetre below this. The actual internal volume of the apparatus when the mercury is standing at any height above H is then easily ascertained.

A sample of gas for analysis is taken at D by means of the pipette P, of about 100 c.c. capacity. This is attached at D, and the air from the intervening tube removed by using the pipette and its reservoir as a mercury pump, the gas removed being swept out of the three-way tap O. After this process has been completed, the reservoir of the pipette is lowered and the taps D and O are opened; gas flows from A to P, and as soon as no more gas passes over, the taps are closed and the pipette disconnected. The sample can then readily be brought into a gas analysis apparatus. The total error in the measurement of the volume need not exceed 0·5 per cent.

The space above the liquid in the fermentation flask is kept as small

as possible, and should not be more than 100-200 c.c. when 1 litre of liquid is employed. The composition of the gas remaining in this space can be ascertained with sufficient accuracy by rapidly removing a sample with the pipette from N; the slight error due to evolution of carbon dioxide or other gas from the liquid in the flask during this process being as a rule negligible. Finally, in calculating the volume of gas actually evolved during the experiment, it is necessary to allow for the volume of gas originally present in the flask; for the gas dissolved in the liquid in the flask; and for the gas corresponding with any residual pressure in the apparatus at the commencement of the fermentation.

THE CLINICAL APPLICATION OF ERGOTAMINE (TYRAMINE)

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Ergotamine, also known as tyramine, is an organic base which can be produced from tyrosine by the action of certain bacteria, normally existing in the alimentary canal.[1] Recent writers[2] have shown that p. hydroxyphenylethylamine occurs in placental extracts, being probably formed by incipient putrefaction, and that this substance, together with other less active bases, is responsible for the pressor action which these substances produce when injected. Barger and Dale[3] have proved that p. hydroxyphenylethylamine is responsible for nearly the whole of the pressor action of the liquid extract of ergot.

Dale and Dixon[4] thoroughly investigated the action upon animal tissues of p. hydroxyphenylethylamine. The drug was found to produce a marked rise of blood pressure when injected intravenously into a pithed animal; this rise was produced partly by augmentations of the cardiac output, and partly by constriction of the arterioles. When injected into a normal animal the drug produced reflex inhibition of the heart through the vagi. The writers conclude that the action of the drug is very similar to that of adrenaline, but that the pressor action is slower and more sustained, whilst its local action is less intense. These differences are of great clinical importance, for owing to its feeble local action, the drug will produce its effects when administered either hypodermically or by the alimentary canal. Adrenaline on the contrary, as is well known, is destroyed in the stomach, and even when injected subcutaneously it is,

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3. *Journal of Phys., Proc. of Phys. Soc.*, May 15, 1909.
4. *Journal of Phys.*, p. 25, 1909.

in the majority of cases, oxidised locally without inducing systemic effects. These conclusions suggested that the drug might be of great clinical importance in the treatment of shock. Dale and Dixon[1] record one observation showing the action of the drug upon man. Ten milligrams of p. hydroxyphenylethylamine were given by mouth, and the blood pressure rose from 116 mm. to 149 mm. in fifteen minutes; after one hour the blood pressure still remained at 134 mm.

At the suggestion of Dr. Dixon the writer made observations to determine more fully the action of the drug when administered to man. The observations fall into three series:—

1. Effect of tyramine administered by mouth to healthy subjects.
2. Effect of tyramine injected hypodermically into healthy subjects.
3. Effect of tyramine administered to patients in the condition of shock or collapse.

The earliest experiments were made upon the writer; later the effects of the drug were tried upon hospital patients resting in bed after minor operations, the subjects being healthy young men between the ages of twenty and thirty. After the correct dose had been ascertained, the drug was used in certain cases of collapse. The subjects were always either lying in bed or else resting in a chair whilst observations were recorded. The blood pressure was measured by Martin's Riva Rocci sphygmometer.

I. A first series of observations was made to ascertain the effect of tyramine administered by mouth on healthy people. The drug was always taken upon an empty stomach. The experiments may be summarised as follows:—

Expt.	Subject	Dose	Blood pressure at 20 min. intervals after administration of drug									
			0	20	40	60	80	100	120	140	160	180
I ...	A.J.C.	15 mgm.	105	105	100	103	103	—	—	—	—	—
II ...	A.J.C.	30 mgm.	102	105	107	107	113	112	—	117	117	110
III ...	H.D.	30 mgm.	110	118	116	116	—	—	—	—	—	—
IV ...	H.D.	50 mgm.	110	118	120	118	—	120	120	—	—	112

A final experiment was made with a larger dose, two doses of 100 mgm. each being given by mouth; a record of the blood pressure of the subject was obtained on the preceding day to serve as a control.

1. *Loc. cit.*, p. 42.

Experiment V.—Subject A.M.

Time	10.30 a.m.	11	11.5	11.20	11.40	11.45	12 noon	12.10			
B.P.	105	105		107	105		103				
			100 mgm. tyramine administered			100 mgm. tyramine administered		dinner			
B.P. on previous day	105	104		—	102		102	„			
Time	12.30 p.m.	12.40	1.40	2	2.20	2.30	2.30	3.30	4.0	5.30	6.30
B.P.	103	105	109	109	107	109		118	tea		113
							Got up for about one hour				
B.P. on previous day	101	—	—	105	—	—	„	105		111	107

From these results it will be seen that in nearly every case the administration of tyramine by mouth was followed by a slight rise of blood pressure. The rise was in all cases slight, and sometimes did not occur until one or two hours after the drug was taken; the rise persisted for some hours after it had commenced. A dose of 200 mgm. of tyramine did not produce any large rise of blood pressure. It will be seen that these results do not agree with the observations recorded by Dale and Dixon in their single experiment upon man, for they obtained a well-marked rise in blood pressure after 10 mgm. of tyramine had been taken: this result was probably due to some idiosyncrasy of the subject for the drug.

II. The effect of injecting the drug hypodermically into healthy subjects was next investigated. The site chosen for injection was found to be of importance. In all cases a marked local reaction followed the injection, but when the drug was injected into the arm or forearm little general reaction followed. The loose tissue around the clavicle was found to be the most favourable spot for hypodermic injection, and the drug was given dissolved in 2-3 c.c. of cold sterile water. Pallor and 'goose flesh' at once appeared around the site of injection, and after a few minutes the pale area was surrounded by a ring of hyperaemic tissue. The following systemic effects were observed: a marked rise of blood pressure, attended in some cases by slowing of the pulse. In some cases the subject mentioned feeling 'throbbing of the heart, and fulness in the head.' The observations recorded are as follows:—

Experiment I.—Subject A.J.C. 15 mgm. tyramine injected subcutaneously. After five minutes the blood pressure rose from 102 to 108; after fifteen minutes more it sank again to 102.

Experiment II.—Subject H.H. 20 mgm. tyramine injected subcutaneously. The blood pressure rose immediately from 124 to 140, and after five minutes sank to 130; after fifteen minutes more it sank again to 124. The pulse was unaltered.

Experiment III.—Subject A.H. 30 mgm. tyramine injected subcutaneously. After five minutes the blood pressure commenced to rise; after ten minutes it had risen from 122 to 138; after ten minutes more it sank again to 122. The pulse rate was unaltered.

Experiment IV.—Subject A.M. 40 mgm. tyramine injected subcutaneously.

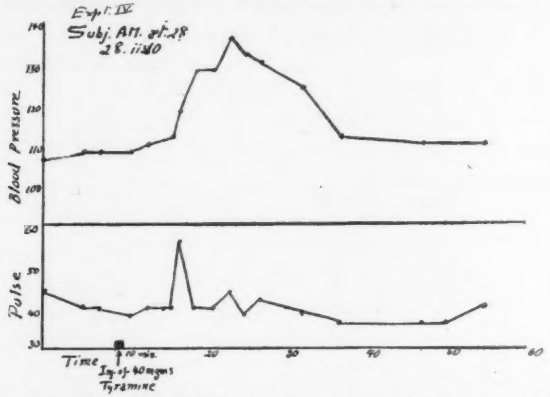


FIG. 1. *Experiment IV.*—Subject A.M., age 28. Record of pulse and blood pressure. Effect of 40 mgm. tyramine injected subcutaneously.

As will be seen from the chart, the blood pressure commenced to rise after six minutes, and after thirteen minutes rose from 108 to 136 mm., and then quickly fell. In this case the pulse increased in frequency when the blood pressure commenced to rise.

Experiment V.—Subject A.U. 50 mgm. tyramine injected subcutaneously.

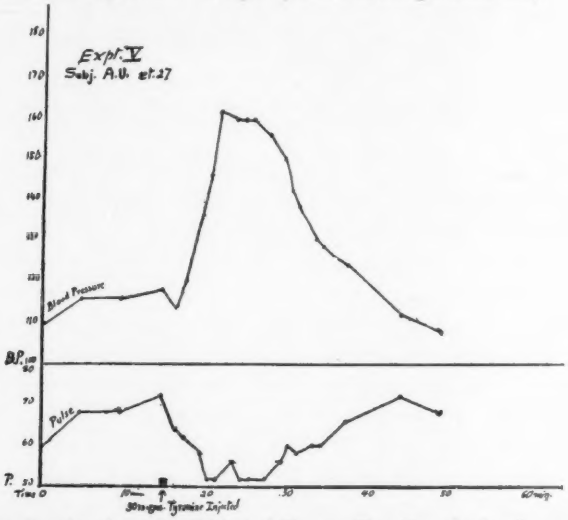


FIG. 2. *Experiment V.*—Subject A.U., age 27. Record of pulse and blood pressure. Effect of 50 mgms. tyramine injected subcutaneously.

The subject of this experiment (A. M.) was also the subject in Experiment V in the first series. As will be seen, 200 mgm. of tyramine when given by mouth only caused a slight rise in blood pressure, whereas 40 mgm., given subcutaneously, produced a very marked reaction.

This dose of the drug caused a very marked rise in the blood pressure, and this was accompanied by considerable diminution in the frequency of the pulse.

Experiment VI.—Subject W.S. 60 mgm. tyramine injected subcutaneously.

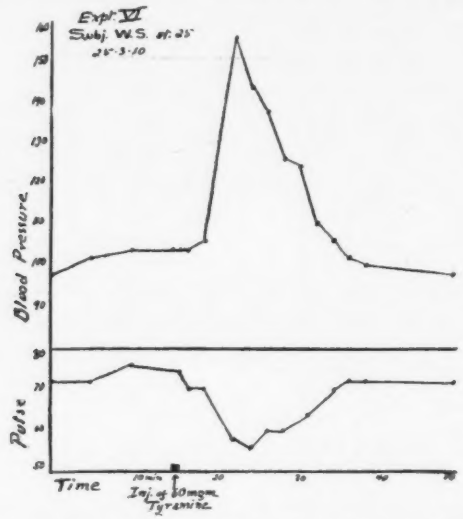


FIG. 3. *Experiment VI.*—Subject W.S., age 25. Record of pulse and blood pressure. Effect of 60 mgm. tyramine injected hypodermically.

In this case the rise in blood pressure was very sudden and very great; the diminished frequency of the pulse during the rise in blood pressure was well marked.

From these observations it was concluded that the most suitable dose of tyramine for clinical purposes was 20-60 mgm., dissolved in 2-3 c.c. of water, and injected subcutaneously into the more connective tissue around the clavicle. As is shown by the observations recorded above, this dose will produce a great rise in the blood pressure in a healthy subject. In no case was the frequency of the pulse much increased; on the contrary, in those cases in which the rise of blood pressure was most marked there was a diminution in the frequency of the pulse. From

the work of Dale and Dixon[1] it appears that the rise in blood pressure observed is chiefly due to constriction of the arterioles; the diminution in the frequency of the pulse is probably due to stimulation of the vagi.

III. The drug was administered to a few patients in a state of collapse; in three cases it was possible to obtain records of the state of the blood pressure after administering the drug.

Case (i). Collapse following trephining and exploration of the brain for a cerebral abscess. Here the blood pressure was 86 mm. Three doses of 20 mgm. of tyramine were given by mouth; the drug had no apparent effect, and the blood pressure continued to sink slowly without interruption.

Case (ii). Collapse following ruptured gastric ulcer. At 11.30 p.m. the ulcer ruptured, and at 4 p.m. next day laparotomy was performed and the ulcer sutured, the operation lasting forty-five minutes. After the operation the patient suffered from severe shock and was treated with saline injections; these had no apparent effect, and tyramine was administered.

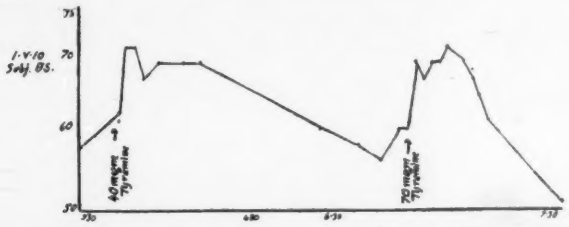


FIG. 4. Subject B.S., age 25. Patient in condition of collapse. Effect of doses of 40 mgm. and 70 mgm. tyramine injected hypodermically.

As will be seen from the appended chart the injection of tyramine was twice followed by a slight rise in blood pressure, with slight general improvement; the effect, however, was only temporary, and at 11.50 p.m. the patient died.

Case (iii) Collapse following haemorrhage from a septic wound. At 8.20 a.m. the haemorrhage occurred.

11.0 a.m.	P. 140	B.P. 100
12.20 midday	P. 160	B.P. 100
12.24	50 mgm. tyramine injected hypodermically	
12.27	P. 160	B.P. 110
12.30		B.P. 112
12.35		B.P. 100

1. *Loc. cit.*, pp. 29-31.

The patient died three days later.

In this case it will be seen that there was a slight but transient rise in blood pressure following the administration of the drug.

Further clinical evidence is required before pronouncing finally upon the value of tyramine in the treatment of shock. From the few cases recorded it appears that tyramine, when injected hypodermically, will produce a slight rise in blood pressure, even in cases of extreme collapse; the drug may therefore prove useful in the treatment of such cases.

Conclusions

1. Tyramine, when given by the mouth to a healthy subject, in doses from 30-100 mgm., causes a slight rise in blood pressure; the rise lasts for some hours.

2. Tyramine, when injected hypodermically into a healthy subject, in doses from 20-50 mgm., causes a rapid and well-marked rise in the blood pressure, lasting for about twenty minutes.

3. Tyramine, when injected hypodermically in doses of 20-50 mgm., into patients in a condition of shock, causes a slight rise in blood pressure.

CHART ILLUSTRATING THE RELATION BETWE

CONCENTRATION

The Phthalein Indicators

4

e Mixture Indicators

5

"Indicateurs du Point Tournant Alcalin"

ARIN | NEUTRAL-RED | PHENOL-PHTHALEIN

HENOL

ROSOLIC ACID

THYMOL-PHTHALEIN

AZOLITMIN)

∝ NAPTHOL PHTHALEIN

GEN ION-CONCENTRATION P_H^+

14

13

12

11

10

$NaOH + NaH_2BO_3$

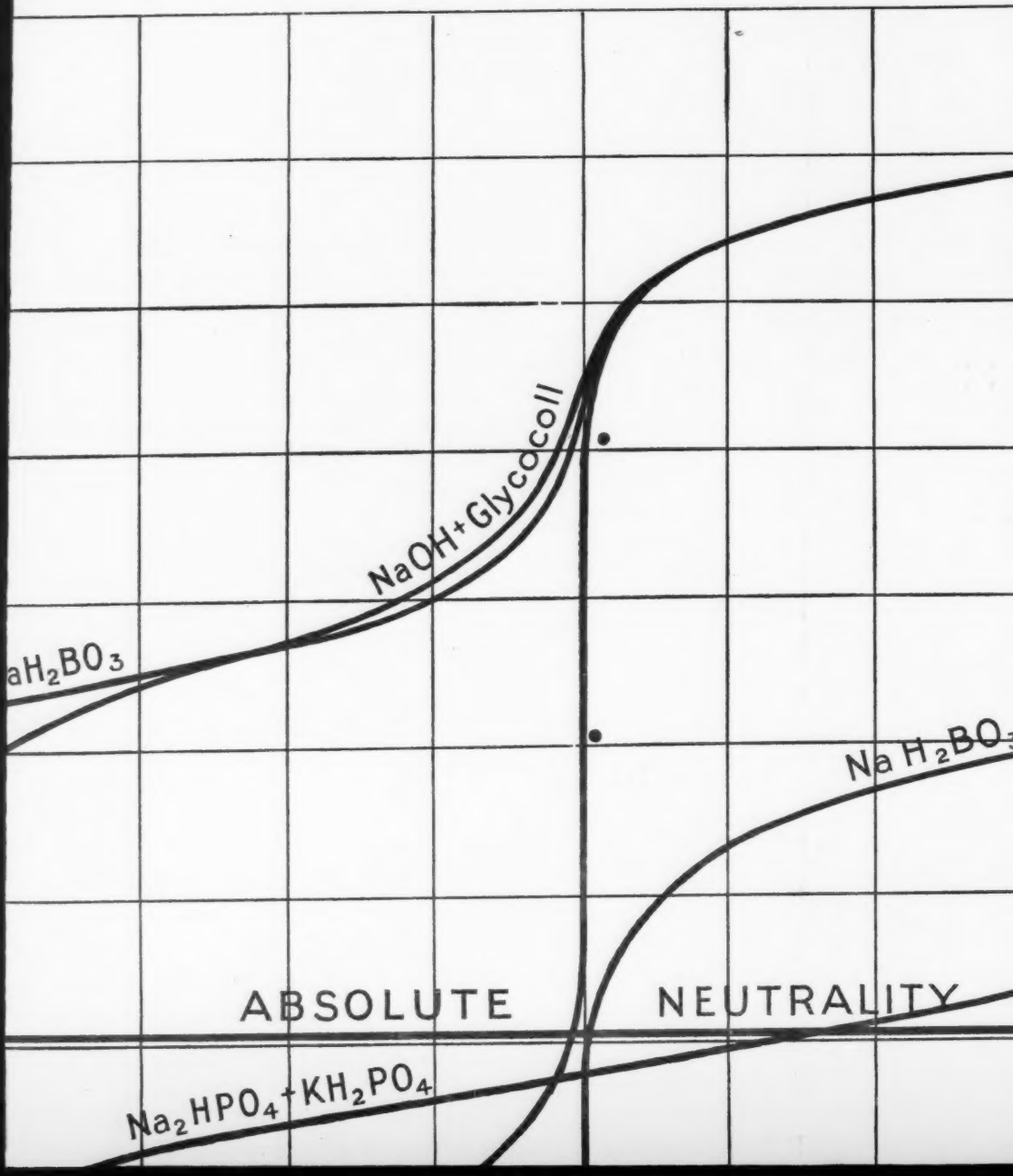
9

8

7

Na

N BETWEEN THE COMPOSITIONS OF CERTAIN MIXTURES, T
NTRATION AND THE RELATED COLOUR CHANGES OF CERTA



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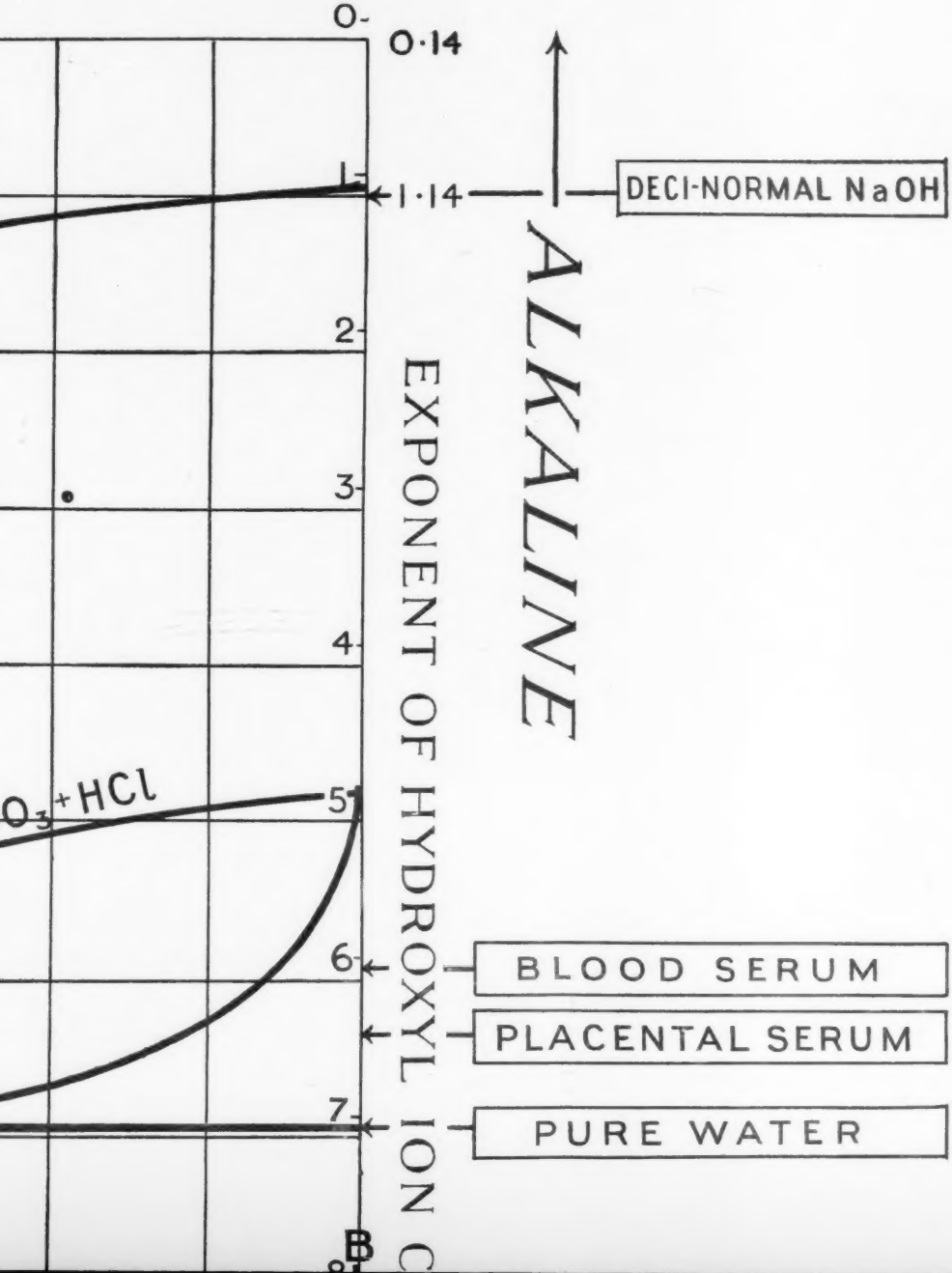
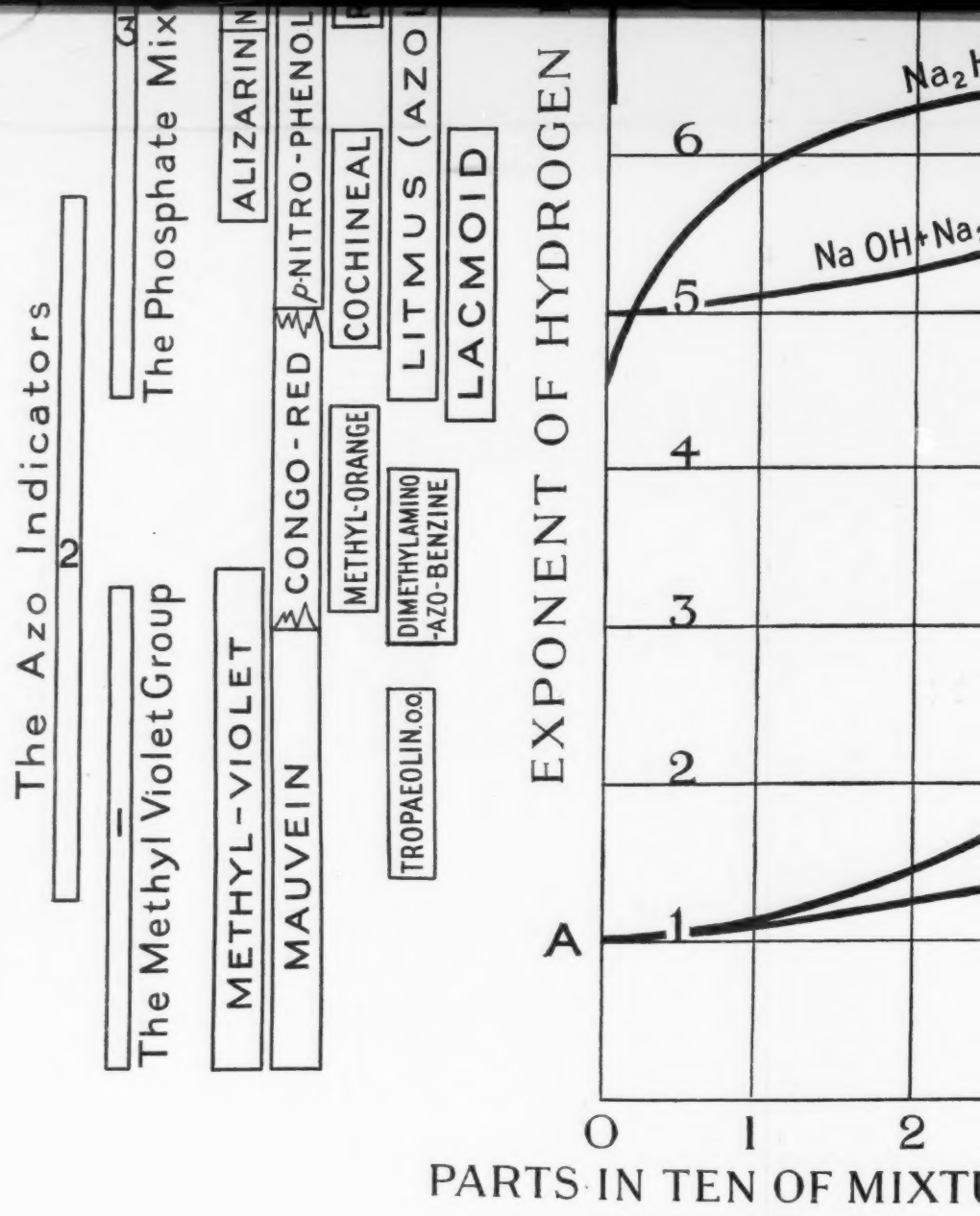
RTAIN INDICATORS





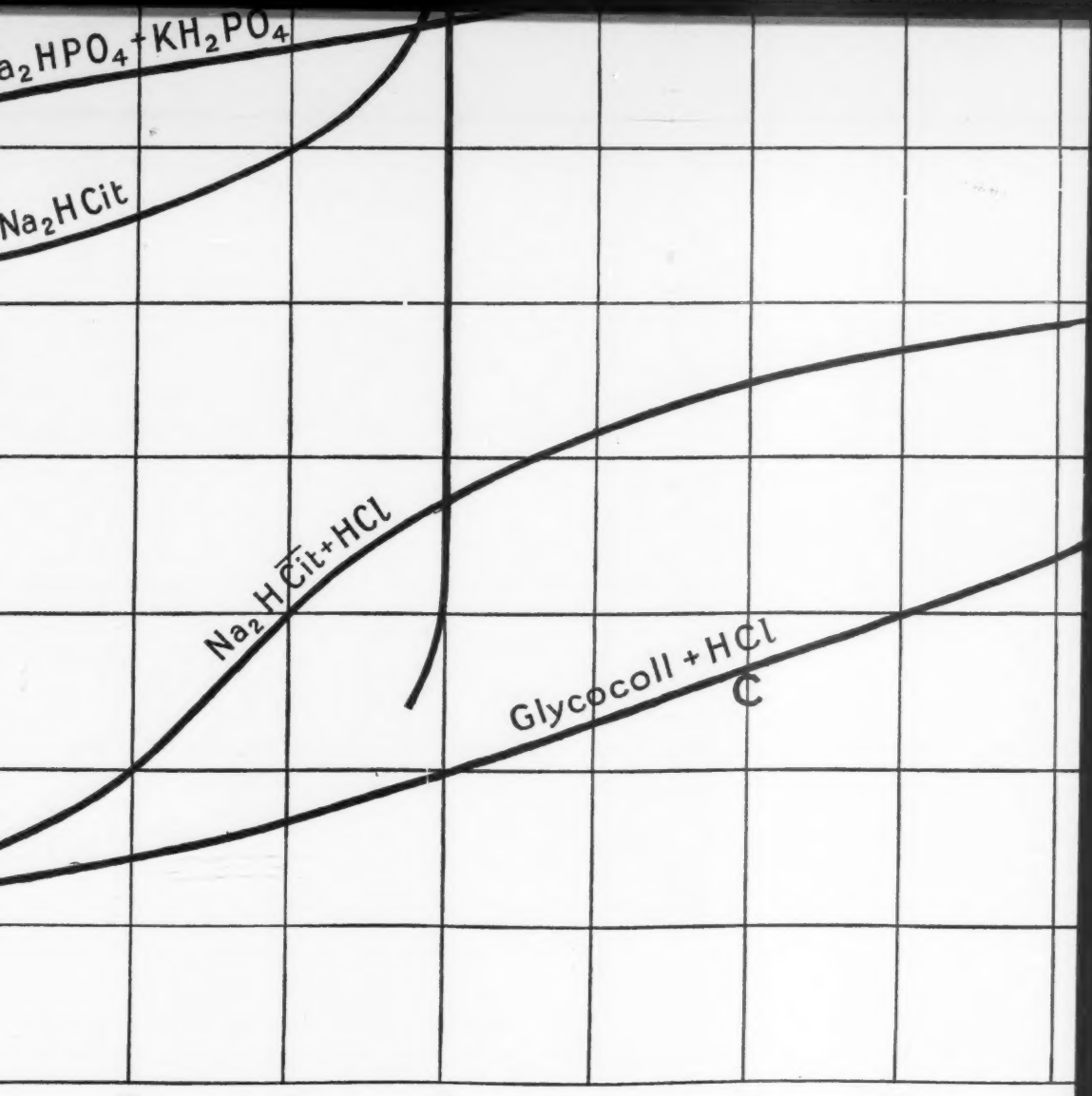
(1) Decinormal hydrochloric acid, designated in the drawing simply ' HCl.'

(2) Decinormal caustic soda, ' NaOH.'

(3) A decinormal solution of glycocoll containing in 1 litre 7·505 grams glycocoll ' glycocoll.'

(4) 1/15 molecular primary potassium phosphate containing in 1 litre 9·078 grams

This Chart is compiled mainly from the curves and tables of S. P. L. Sörensen, Cor Placental Serum—Jankowsky and Hö



TURE OF THE 'MORE ALKALINE' OR 'LESS ACID' C

ocoll and 5·85 grams sodium chloride,

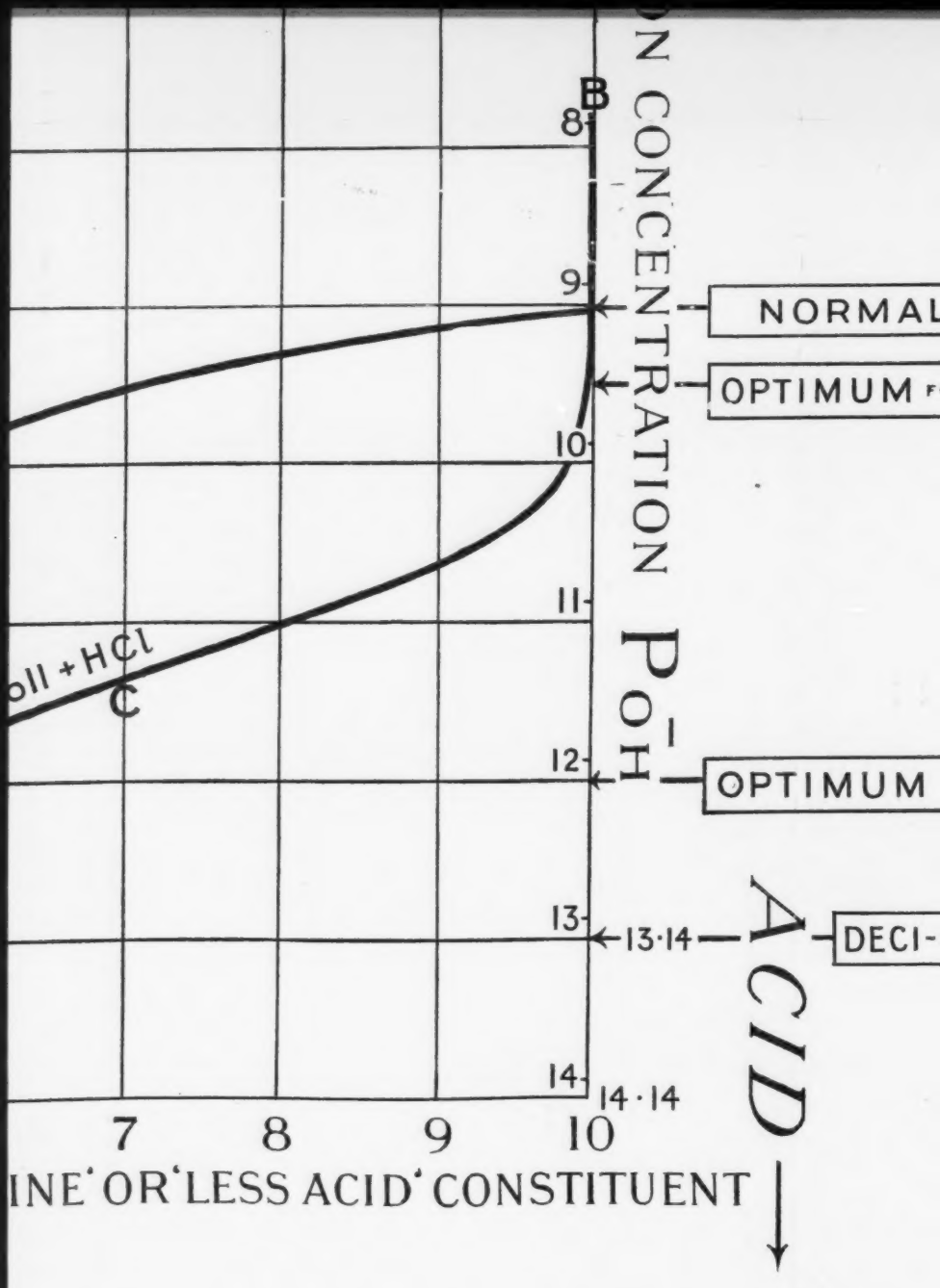
rams, 'KH_2PO_4.'

(5) 1/15 molecular secondary sodium phosphate cont

(6) 1/10 molecular secondary sodium citrate containi
of normal caustic soda, 'Na_2HCit.'

(7) A solution containing in 1 litre 12·404 grams bor

Comptes Rendues des Travaux du Laboratoire de Carlsberg, Copenhagen, VIII,, pp. 1-168, 1909 ;
Höber ; Normal Urine—Walther Löb and Shigeji Higuchi ; Biochem. Zeitschr. XXIV, 92-107,



molecular secondary sodium phosphate containing in 1 litre 11·876 grams, 'Na_2HPO_4.'

molecular secondary sodium citrate containing in 1 litre 21·008 grams hydrated citric acid a
of normal caustic soda, 'Na_2HCit.'

olution containing in 1 litre 12·404 grams boric acid and 100 c.c. of normal caustic soda, 'NaH

rg, Copenhagen, VIII,, pp. 1-168, 1909 ; Blood Serum—Höber- Pflüger Archiv., LXX
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AL URINE

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NaH_2BO_3.'

XXXI, 522, 1900

THE ACTION OF SOME ISOQUINOLINE DERIVATIVES

By P. P. LAIDLAW, M.A., B.C.

From the Wellcome Physiological Research Laboratories, Brockwell Hall, Herne Hill, S.E.

(Received July 22nd, 1910)

Our knowledge of the fairly extensive series of iso-quinoline derivatives has recently been considerably augmented by Pyman.[1] He undertook the study of the oxidative decomposition of laudanosine with the object of producing 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride, which, in view of its close chemical relationships to hydrastinine and cotarnine, was expected to have a similar physiological action. The work was continued along lines opened up by the original investigation, and a number of other new substances were made. These new substances have been examined with a view to determining their pharmacological action, and although the examination cannot be considered as exhaustive, sufficient has been done to suggest an interesting relationship between chemical structure and physiological action. This relationship is still further emphasised when the structure and physiological action of closely related compounds, which have been worked out by other observers, is taken into consideration.

I desire, therefore, to bring forward this relationship in the first part of this paper, and to devote the second part of it to a consideration of the action of one of the new iso-quinoline derivatives which appears to possess features which may render it of use in therapeutics, namely, 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride.

Part I

The action which is characteristic of a large number of iso-quinoline alkaloids is that of producing a condition of increased reflex excitability. The degree of hyperexcitability induced by these substances varies within

wide limits. The effect is comparable in many instances with that of strychnine. It is this strychnine-like effect which appears to bear a definite relationship to chemical structure.

There are three series of compounds corresponding to three series of alkaloids which illustrate the relationship:—

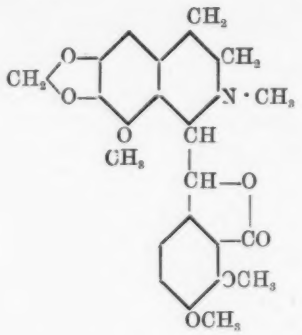
I. Narcotine and its derivatives.

II. Hydrastine and its derivatives.

III. Laudanosine and its derivatives. (This series may also be regarded as Papaverine derivatives.)

The first two have been worked out for the most part by other observers, and with one or two exceptions I have relied entirely on their statements. Where no name is quoted the observation is original, although not necessarily new.

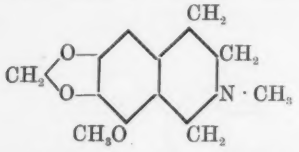
The animals used for my investigations were frogs and guinea-pigs. Both of these readily show a characteristic picture with pharmacological agents of this nature, and moreover, small doses are as a rule sufficient to elicit the characteristic effect in them. Narcotine, amongst other opium alkaloids, has long been known to produce a strychnine-like effect on the spinal cord (von Schroeder,[2] Stockmann and Dott,[4] etc.). It has the constitutional formula



and is the parent of the first series of alkaloids we will consider. It is readily decomposed into opianic acid and cotarnine, and from the latter a series of compounds have been made which produce convulsant effects when administered to animals.

Hydrocotarnine, which occurs in small quantity in opium, was shown by Falck,[3] Stockmann and Dott[4] to produce strychnine-like effects. It

is 8-methoxy 6:7-methylene-dioxy 2-methyl-tetrahydro-iso-quinoline.

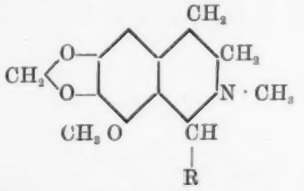


It has been shown by Heinz,[5] who worked with material prepared by Freund and Reitz, that the four following derivatives—

1-ethyl hydrocotarnine,
1-propyl hydrocotarnine,
1-benzyl hydrocotarnine,
1-phenyl hydrocotarnine,

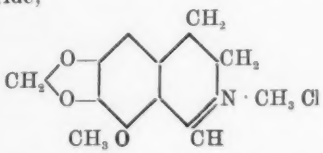
were convulsant poisons of the strychnine class. The ethyl and propyl derivatives are particularly potent in this respect.

This series of compounds which produce the characteristic state of hyperexcitability culminating in convulsions have the general formula



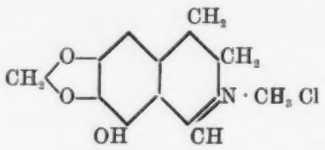
where R is an opianyl, benzyl, phenyl, propyl, ethyl group or merely hydrogen. Anhydrocotarnine acetone also produces a condition of greatly increased reflex excitability in frogs and guinea-pigs, which, in view of this generalisation, would indicate that it reacts as 1-acetonyl-hydrocotarnine.

If, now, R is removed and the substance is no longer a tetrahydro-iso-quinoline derivative, this strychnine-like action disappears, e.g., cotarnine, or 8-methoxy 6:7-methylene-dioxy 2-methyl 3:4-dihydro-iso-quinolinium chloride,



has a purely depressant effect on the central nervous system, and never produces increased reflex irritability. The same is true of the

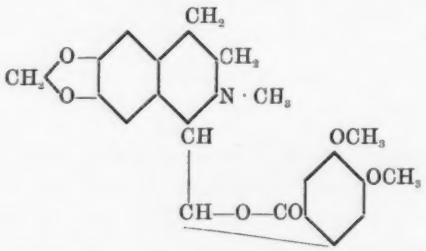
chloride of cotarnamic acid, 8-hydroxy 6:7-methylene-dioxy 2-methyl 3:4 dihydro-iso-quinolinium chloride



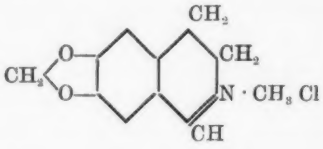
In the frog some stiffness in movements is noticeable after some hours, but convulsions do not supervene, and in the guinea-pig the effect is purely depressant.

Turning now to hydrastine, we find the same relationship, but there are not so many examples illustrative of it.

Hydrastine itself was shown by Falk,[6] Marfori,[7] Cerna,[8] Pembrey and Phillips,[9] to produce a strychnine-like effect. It is narcotine without a methoxy group in the 8 position of the iso-quinoline ring.

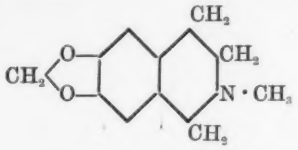


Hydrastinine chloride, the homologue of cotarnine chloride and prepared by analagous methods, has been shown to be devoid of a strychnine-like effect. (Marfori,[7] Falk,[6 & 12] etc.)

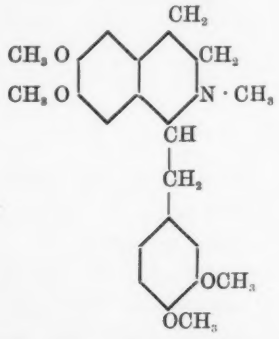


Like cotarnine, it has a purely depressant effect on the central nervous system, and causes death by respiratory failure. On the other hand,

hydro-hydrastinine (cf. hydrocotarnine) possesses a characteristic strychnine-like action in a high degree (Kramm[10]).

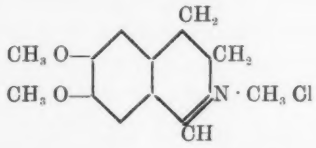


In the laudanosine series a similar relationship obtains. Laudanosine itself was shown by Babel[11] to produce a typical condition of hyperexcitability. Reference to the structural formulae will show its close relationship to narcotine and hydrastinine.



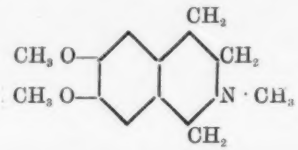
Pyman[1] showed that this alkaloid was susceptible of oxidative decomposition along lines analogous to those producing cotarnine and hydrastinine from narcotine and hydrastine respectively.

The substance 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride analogous to cotarnine and hydrastinine is incapable of producing exaggeration of reflexes.

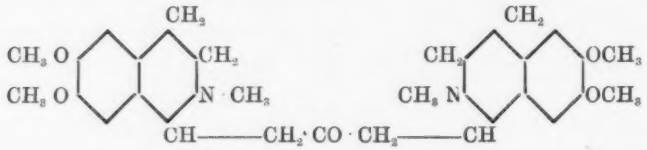


Its general effect is depressant to the central nervous system, and it is strictly comparable in this respect to its allies cotarnine and hydrastinine. The corresponding 2-ethyl compound is precisely similar in action to the

2-methyl compound. On the other hand, 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline induces a typical strychnine-like effect. (Compare with hydro-hydrastinine and hydrocotarnine.)



The only other tetrahydro-iso-quinoline compound in this series that I have tested is acetonyl bis (6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline).

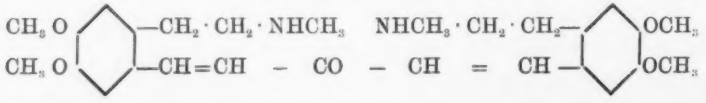


This substance also produces a condition resembling mild strychnine poisoning in frogs and guinea-pigs. This effect is not nearly so striking as in the case of anhydrocotarnine acetone.

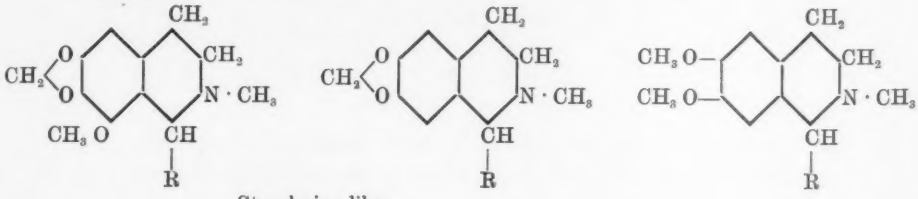
The degree of exaggeration of reflexes induced by these various tetrahydro-iso-quinoline compounds varies considerably. It would require careful comparison of the whole series to prove whether their potency bore any relationship to their composition. It is worth while noting, however, that hydrocotarnine, hydro-hydrastinine and 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline are intense convulsants. Anhydrocotarnine acetone is very potent, and the 1-ethyl and 1-propyl hydrocotarnines are reputed by Heinz to be very active in this respect. The smaller substituting group in the 1 position appears to increase this activity. The larger substituting groups seems to be more depressant to the higher centres (narcotine, laudanosine).

Summing up for these iso-quinoline derivatives, we may say that N-methyl-iso-quinoline derivatives substituted in the 6:7:8 positions

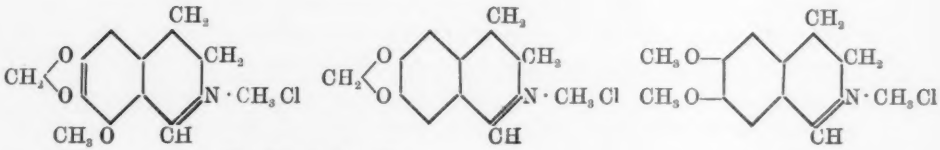
* It is equally possible that the formula of this compound is



with methoxy or methylene-dioxy groups, have or have not strychnine-like actions according as they are tetrahydro-iso-quinoline derivatives or not.

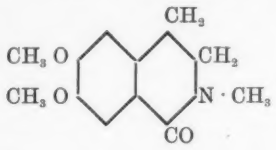


Strychnine-like.

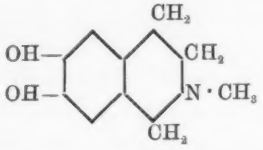


Not Strychnine-like.

A number of other compounds belonging to the laudanosine series have been investigated in connection with their power of inducing hyperexcitability. The introduction of a keto group to the 1 position abolishes the strychnine-like effect in the case of 1-keto 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline. Although some stiffness of movement is noticeable in the frog, after large doses, similar to early strychnine poisoning, in the guinea-pig the effect is purely depressant.

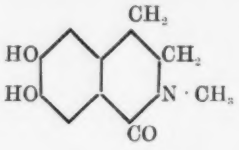


Replacement of the methoxy groups in 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline by hydroxyl groups abolishes the power of inducing hyperexcitability.

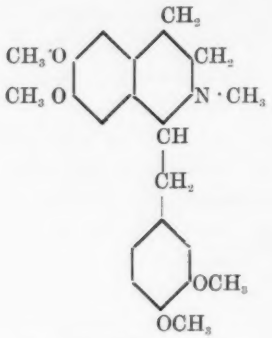


6:7-dihydroxy 1-keto 2-methyl-tetrahydro-iso-quinoline is also inactive in

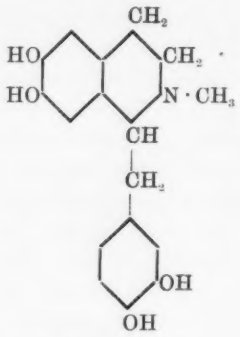
this respect. (Some stiffness of movement is noticeable in the frog.)



Replacement of methoxy groups by hydroxyl groups again abolishes the strychnine-like effect in the case of laudanosine or N-methyl-tetrahydro-papaverine, N-methyl-tetrahydro-papaveroline being devoid of this effect when tested upon both frogs and guinea-pigs. This compound, which has not previously been described, was prepared by Pyman by the action of hydrochloric acid at 170° on laudanosine. The hydroxy compounds are more susceptible of oxidation, and in alkaline solution are readily decomposed. This may to some extent explain the different behaviour of these substances.



N-methyl-tetrahydro-papaverine.
Convulsant

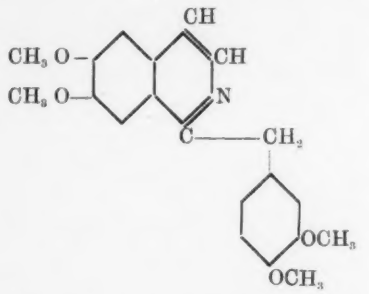


N-methyl-tetrahydro-papaveroline.
Not convulsant.

The previous series of compounds are all substituted N-methyl-iso-quinoline derivatives. There are a number of other convulsant poisons which also possess an iso-quinoline nucleus; for example, papaverine (von Schroeder, Babel). These have not been studied in such detail, as the number of known substances is not so large.

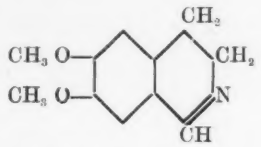
Comparison of those which have come under my notice suggests again a relationship between chemical structure and pharmacological

action. Papaverine produces a stage of reflex hyperexcitability followed by depression in the mammal and coma in the frog.

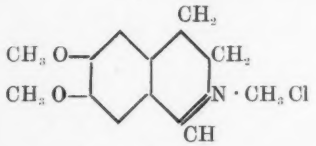


The introduction of a methyl group in relation to the nitrogen abolishes this effect. Papaverine metho-chloride produces a few muscular twitches and then coma in the frog. It produces depression and a few muscular twitches in the guinea-pig. At no stage during onset or recovery from the action of the drug is there any sign of a strychnine-like effect. It thus falls into line with our law with regard to 6:7-dimethoxy 2-methyl-iso-quinoline derivatives (see above). When this is reduced to the tetrahydro derivative (laudanosine) the strychnine-like action reappears.

A similar relationship obtains in the simpler compound 6:7-dimethoxy 3:4-dihydro-iso-quinoline. This substance resembles hydrocotarnine in its strychnine-like action.

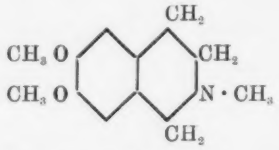


Methylate the nitrogen and this property disappears.



6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride.

Reduce this to 6:7-dimethoxy 2-methyl-tetrahydro-iso-quinoline and the strychnine-like effect reappears in full strength.

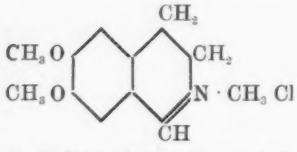


It is thus evident that a different law prevails in the case of iso-quinoline derivatives without a methyl group on the nitrogen, but sufficient examples are not available for a generalisation.

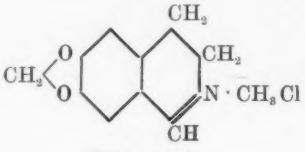
It seems clear, however, if the compounds containing hydroxy groups or keto groups are excepted, that one generalisation will cover all these iso-quinoline alkaloids. Iso-quinoline compounds substituted in 6:7:8 positions with methoxy or methylene-dioxy groups are convulsant when the nitrogen is trivalent, and are devoid of this effect when the nitrogen is pentavalent.

Part II

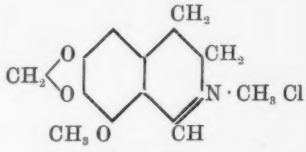
The alkaloid which forms the subject of this part of the paper is 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride. As will be seen from the structural formulae below, it has close chemical relationships with hydrastinine and cotarnine. These two alkaloids have for long enjoyed a reputation in therapeutics as haemostatics, particularly in abnormal uterine conditions. The similarity in chemical constitution suggests a similarity in physiological action. This would seem to be the more probable when the similar action of the parent alkaloids and other derivatives from them is taken into account. Reference to the earlier part of this paper will make this point clearer. The close relationship between the new alkaloid and hydrastinine and cotarnine is well shown in their structural formulae.



6:7-diamethoxy 2 methyl 3:4 dihydro iso-quinolinium chloride.



Hydrastinine chloride.



Cotarnine chloride.

It was therefore considered advisable to undertake a comparative investigation of the three substances. This appeared to be the more desirable since it was soon clear that differences in action between these three compounds were for the most part differences of degree and not of kind.

Previous investigators have unfortunately obtained very contradictory results when working with hydrastinine or cotarnine. This necessitated the conduction of a number of experiments with a view to ascertaining their mode of action with greater certainty, and if possible reconciling the divergent views of earlier workers. A number of experiments were also undertaken to determine the action of 6:7-dimethoxy 2-ethyl 3:4-dihydro-iso-quinolinium chloride. It was found that in most respects its action did not differ materially from that of the corresponding 2-methyl compound. But it is considerably more toxic.

6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride was given hypodermically in varying doses to frogs and guinea-pigs. Very little can be made out by this means of investigation beyond the two facts that it is depressant to the nervous system and that the cause of death in the mammal is failure of the respiration. When 50 mgm. doses of the alkaloid are given to guinea-pigs (about 700 grams) no abnormal symptoms are noticeable for the first twenty or thirty minutes. At the end of this time there is usually observable a few signs of depression, such as disinclination to move, cessation of feeding, etc. When the animal is disturbed, however, the movements are well co-ordinated and apparently normal.

With larger doses, 100 mgm. for a guinea-pig of about the same weight, the depression becomes much more obvious, and towards the end of the first hour succeeding the administration of the alkaloid the respiration becomes slower and is slightly laboured. This dose is sometimes fatal, and it is noticeable that the respiration becomes gradually slower and more laboured and then intermittent. The heart continues to beat vigorously until the respiration is failing. With the failure of respiration the heart beat becomes weaker, and a few convulsive movements ensue and the animal dies.

A guinea-pig survived three subcutaneous doses of 75 mgm. of the alkaloid in twenty-four hours. It is therefore either readily destroyed or rapidly excreted.

The descriptions of the action of cotarnine or hydrastinine on the

intact mammal, as given by various authors (Falk, Marfori, von Bunge, Ronsse, etc.) are much the same as the above. A few experiments were made with cotarnine, and essentially the same clinical picture was seen with toxic doses; but cotarnine appeared to be less toxic. Doses of 150 mgm. of cotarnine were found to be fatal for guinea-pigs of about 700 grams, but 100 mgm. doses were not. The heart beat also did not appear to be quite as well maintained during the later stages of poisoning by this substance. On the central nervous system these three alkaloids exert a depressant action, and cause death by respiratory failure. The failure of the respiration is of central origin, since there is no evidence of any well-marked peripheral action by these three substances (see later).

The vascular system. A series of experiments were performed with a view to determine the action of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride on the vascular system. These were for the most part performed upon pithed cats, but a number were also performed upon anaesthetised animals. Chloroform, ether, paraldehyde and ether, and urethane, were the anaesthetics employed. The main features of the results obtained upon cats were confirmed upon rabbits.

When a dose of 10 mgm. of the new alkaloid is injected into the blood stream of a pithed cat, and the blood pressure is recorded by a mercury manometer from the carotid artery, there is noticeable a rise of blood pressure. At the same time as the blood pressure rises the heart beat becomes slower and the oscillations of the mercury manometer increase in size. With larger doses, 20 mgm., the same action is observable, but both are slightly more marked. Considerable variation is met with in the height of the rise of blood pressure. It is never very large, and rarely exceeds 40 mm. of mercury with doses of 10-20 mgm. Fig. 1 shows one of the smaller rises of blood pressure; others will be seen in other figures. The rise in blood pressure is the result of two factors—(1) slight vaso constriction, (2) increased cardiac output.

The rise of blood pressure being partly accounted for by increased cardiac activity, and being never very large, it will be obvious that the vaso-constriction must be slight. It was found, indeed, that the vaso-constriction was not easy to demonstrate.

Inspection of the viscera during the administration of a full dose of the alkaloid appeared to show that some constriction of the arterioles occurred, but it was never incontestable except in the case of the highly vascular organs, such as the uterus of a virgin rabbit. In this instance

slight pallor occurred as the result of the administration of the drug, but the experiment is complicated by the fact that the alkaloid causes a contraction of the uterine muscle. In oncometric observations a change of organ volume is sometimes seen, but as a rule the volume remains practically unchanged. It would seem that the rise of blood pressure compensates for the vaso-constriction, and the blood content of the viscus is unaltered. The intestines and kidneys were the viscera employed in making oncometric observations. (See fig. 16.)

In order to eliminate the effect of variations in blood pressure, a number of perfusion experiments were performed upon isolated organs. Slight slowing of the venous outflow was noticeable as the result of injecting 6:7-dimethoxy 2-methyl 3:4 dihydro-iso-quinolinium chloride into the perfusion cannula. Fig. 2 is a drop record of the venous outflow from a rabbit's kidney under the influence of the alkaloid. The effect is small and of short duration. A number of experiments were performed with a perfusion apparatus similar to that employed by Brodie and Dixon[23] when studying the innervation and action of drugs on the vessels of the lungs. Here the venous outflow is collected in a small air-tight receiver, from which it is sucked away by a pump which is capable of very fine adjustment so as to balance exactly the venous outflow. The volume of the fluid in the receiver is recorded by a Brodie bellows recorder. Variations in venous outflow cause variations in the amount of fluid in the receiver, and hence in the record traced by the bellows recorder. In all cases 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride caused a slowing of the venous outflow.

The vaso-constriction is the result of an action of the alkaloid upon the plain muscle of the arterioles, and cannot be attributed to an action on nerve endings or the vaso-motor centre. For the rise of blood pressure is no greater in an anaesthetised animal with the vaso-motor centre intact than in a pithed animal with the vaso-motor centre destroyed; indeed, it is usually less in the former case than in the latter. The effect of the anaesthetic might account in part for this, but the response to the alkaloid was no greater in a high-pithed animal (respiratory and vaso-motor centres intact) than in a low-pithed animal. Moreover, after sufficient ergotoxine had been given to cause complete reversal of the adrenine response (see Dale[21 & 22]), the rise of blood pressure was unimpaired.

In this respect it appeared at first that 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride differed from hydrastinine, for most

observers attribute the vaso-constriction it induces, in part at any rate, to an effect on the vaso-motor centre. Thus Falk[6 & 12] found that hydrastinine produced a constriction of the arterioles, which he attributed in part to an action on the vaso-motor centre, since chloral in large doses diminished but did not abolish the rise in blood pressure induced by this substance. Marfori[7] found that section of the cord in the cervical region in a dog abolished the usual rise of blood pressure induced by hydrastinine. But the protocol of the experiment illustrating this point shows that his animal was in very poor condition after the operation. Ronsse[24] found that chloral had practically no effect on the rise of blood pressure induced by hydrastinine. Pick[13] found a diminution of venous outflow from the femoral and mesenteric veins in a dog as a result of the administration of hydrastinine.

Kurdinowski[18] found no variation in the rate of outflow from a perfused isolated uterus, and therefore attributed its specific constriction effect to a central action. von Bunge,[14] on the other hand, found an increased outflow from the veins of isolated perfused kidneys and diminished outflow from the veins of perfused hind limbs and spleen. He attributed the effect of hydrastinine to an action on a peripheral nervous mechanism and not on the muscle, because he found that the venous outflow from an organ which was removed from the body and perfused at once, was diminished when hydrastinine was added to the perfusion fluid, while the venous outflow from the organ which was perfused some hours after death of the animal, was increased when hydrastinine was administered. In his view the nerve or nerve-ending died before the muscle, and this explained the difference in the result of the two cases. In this position he stands alone, and the evidence in his favour is not of the best.

In view of this conflicting evidence, a number of experiments were made with hydrastinine, and it was found that its action on the vessels is almost identical with that of 6:7-dimethoxy 2-methyl 3-4-dihydro-iso-quinolinium chloride.

It produces a rise of blood-pressure of about the same height as that of the new alkaloid. As will be seen later, this is partly explained by increased cardiac output. It produces a slight vaso constriction and diminished outflow from the kidney vessels (fig. 3). It produces a similar degree of diminished venous outflow from the hind limbs of a cat perfused by the Brodie-Dixon method. The rise of blood-pressure is not greater in animals under anaesthesia with the vaso motor-centre intact than in

pithed animals. A high-pithed animal does not show a better response than a low-pithed one. Finally, after sufficient ergotoxine had been given to reverse completely the adrenine response, the rise of blood-pressure induced as the result of the administration of hydrastinine is unimpaired (fig. 4). The evidence in favour of a central action is not good, and depends for the most part on a supposed selective depressant action of chloral on the vaso motor-centre, and is disproved by the evidence adduced above. The action of hydrastinine is due to a direct effect on the muscle of the arterioles, and is at best a weak one, very similar to that of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride. Cotarnine is usually stated to have no effect on the arterioles, (Falk,[15] Marfori,[16] Ronsse,[24]) and this is in agreement with the results of my experiments. When a dose of this substance is injected intravenously into a pithed cat there is produced a sudden fall of blood-pressure with a slow return to the normal.

Kehrer found in some few cases a slight rise after the primary fall, and he attributes this to a central effect. The amount of the rise appears to have been trivial, and was probably due to some other factor, since it was variable in his experiments and absent in my own. The colour of the viscera remains unchanged under full doses of the substance or in some instances there is a transient flush observable, but this effect is slight. Fig. 5 shows the result of adding cotarnine to the perfusion fluid, which was employed to perfuse the hind limbs of a rabbit. There is practically no alteration in venous outflow. In some instances a slight increase in venous outflow was obtained.

Action on the Heart. The second factor in the rise of blood-pressure is increased cardiac output. When 6:7-dimethoxy 2-methyl 3-4-dihydro-iso-quinolinium chloride is injected into the perfusion cannula which is being employed to perfuse an isolated heart after the Locke-Langendorff method, there is noticeable a slowing of the heart beat accompanied by an increase in force. When cardiometer records are taken of the cardiac output, the results are very similar. Fig. 6 shows such a record from a pithed cat. It is obvious from inspection that the output is increased, and that the rate is perceptibly diminished. Measurement shows that (1) the average output per beat is 41 v. (where v. is a constant depending on the bellows recorder); (2) the heart rate is 35 beats in 10 seconds before administration of the drug. At the point where the maximal effect of the drug occurs—

The average output per beat is 56 v.
The rate is 31 beats in 10 seconds.

Before administration of the alkaloid the output in 10 seconds = 1,431 v., and afterwards the output in 10 seconds = 1,736 v.

In other words, the output per minute is increased by a little more than one-fifth.

Figs. 7 and 8 show the effect of hydrastinine on the isolated heart and on the cardiac output respectively. Measurements from the cardiometer record show:—

Rate before drug	42 beats in 10 seconds.
Average output per beat	14 v.
Rate after drug	37 beats in 10 seconds.
Average output per beat	19 v.
Output per 10 seconds before drug ...	588 v.
Output per 10 seconds after drug	703 v.

In other words, the output per minute is increased by a little less than one-fifth.

In this respect also hydrastinine has a very similar action to that of the new alkaloid.

Cotarnine, on the other hand, does not produce the same effect. The heart becomes slower, but the output remains the same, or may even be diminished (marked decrease in force of heart beat or output per beat is unusual). Fig. 9 shows a fairly typical result. In this tracing before the administration of the drug the average rate of heart per 10 seconds was 32, the average output per beat 12 v.—hence output per 10 seconds = 384 v.

At the maximal effect of the drug (20 mgm. intravenously, 2½ kilos. cat)—

The average rate of heart per 10 seconds was 29·5.
The average output per beat was 11 v.
Hence output per 10 seconds = 324 v.

In other words, the output from the heart under the influence of cotarnine is diminished by about one-fifth.

It is clear that the fall in blood-pressure induced by cotarnine is of cardiac origin. Vaso dilatation may play a part, but if so it must be very slight. The fall of blood-pressure induced by cotarnine preparations is recorded by Heinz[5] to be of cardiac origin. Kehrer also attributed the fall to diminished cardiac activity. Neither appears to have demonstrated the point successfully, however. The effects of hydrastinine and cotarnine

on the vascular system are not of such a kind as would seem to render them valuable therapeutic agents in haemorrhage.

Since the effects of these three substances on the vascular system cannot explain their reputation as haemostatics, it appeared to be possible that they might aid the process of blood coagulation. A number of experiments were therefore conducted to determine the influence of these substances on the clotting of blood-plasma and fibrinogen solutions.

None of them can replace fibrin ferment. In small doses they did not affect the rate of clotting, or, as far as could be determined, the consistency of the clot. In large quantity they delay the rate of clotting. 10 c.c. of oxalate plasma 0·5 c.c. calcium chloride solution, and 4 drops fibrin ferment clotted in ten minutes. An exactly similar test mixture to which sufficient 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride was added to make a 1 in 1,000 solution took thirteen minutes.

Similar results were obtained with hydrastinine and cotarnine. Marfori's[16] statement that cotarnine is unable to hasten the process of blood coagulation is thus confirmed and extended to the two other alkaloids. There still remained the possibility that these substances might have an effect on the rate of blood coagulation when injected into an animal. It was possible that their presence in the circulation might, although incapable of influencing the rate of blood coagulation directly, bring about some difference in the normal equilibrium point indirectly. That is, produce an effect comparable to that produced by peptone, but in the opposite direction. To test this point an anaesthetised rabbit was bled from one carotid artery at intervals into a series of small test-tubes. The coagulation time was determined by timing the point at which the test-tube could be inverted without spilling the contents. This method of determining the coagulation is, of course, crude, but the results are as good as those with more complicated apparatus. It was found that the coagulation time was reduced after the administration of 20 mgms. 6 : 7-dimethoxy 2-methyl 3 : 4-dihydro-iso-quinolinium chloride, from about 6 min. to 1 min. 25 secs. As, however, six small bleedings (2 c.c.) had been made in the fifteen minutes, it was doubtful how far this result is due to the drug and how far to the influence of bleeding, for bleedings shorten coagulation time. A similar series of bleedings was therefore made upon a second rabbit as a control, and it was found that after the first three bleedings from the carotid artery the coagulation times became very irregular, and some were very short, e.g. 1 min. 45 secs. It is very probable that the short times of later bleedings are due to

small quantities of fibrin formed at the cut end of the carotid artery which were swept into the test-tube on collecting the later samples. The method is therefore worthless for making a series of comparative coagulation times.

A method for timing the rate of blood coagulation on small samples was then employed, and blood taken from the ear vein of a rabbit. Here the results were again very irregular in the normal animal. Thus coagulation times as low as fifty-eight seconds are obtained if the blood be taken from a large ear vein and pressure is employed along the vein to aid the expulsion. On the other hand, if the bleeding is taken from a very small superficial vein near the tip of the ear the coagulation time may be nearly four minutes. The results obtained depend on the amount of kinase picked up by the blood during its passage from the vessel to the surface, and this depends on (1) the depth at which the vein lies, (2) whether pressure is used to aid the expulsion of the drop of blood. This method was therefore abandoned since the results obtained are incapable of accurate interpretation. Attempts were also made to measure coagulation times by bleeding a series of samples through a paraffin-coated cannula from the carotid artery. In this case also, clotting started in the cannula at the end of twenty minutes, and the coagulation times became very irregular. These methods having failed to demonstrate any action of the series of alkaloids on the coagulation of the blood, the point was not further investigated. It is clear that none of these substances have a profound influence on the coagulation time of blood since they never cause intravascular clotting even when given in heroic doses

So far the haemostatic effect of these substances is unexplained, but, as will be seen below, these drugs have a definite effect on the uterine musculature, and it is this effect which is probably the source of their reputation in therapeutics. Their rational application appears to be limited to uterine haemorrhage.

Action on the Uterus. On the isolated uterus of cat, rabbit, or guinea-pig, the effect of a few milligrams of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride is to produce a well-marked increase in tonus. The degree of contracture varies considerably with different organs, but the response is always motor.

Fig. 10 shows the effect of 10 mgms. of the new alkaloid on an isolated virgin cat's uterus suspended in 250 c.c. of warm oxygenated

Ringer solution. On this particular uterus a dose of 3 mgms. produced an almost maximal response. Occasionally a poor response is met with, as the result of a dose of 10 mgm. a slight rise of tonus without any alteration in the rhythm may be the only observable effect.

Similar results are seen when similar quantities of hydrastinine are given under these conditions (fig. 13). Kurdinowski[18] found identical results in the case of perfused isolated rabbits' uteri. Cotarnine with small doses (3 mgms.) does not give quite such a good response as the other two; but, in view of the variability in the degree of the response, no great weight can be attached to this point. The effect of 10 mgms. is shown in fig. 12. It is in all essentials similar to the other two. Kehrer[17] found similar effects with cotarnine in the case of the cat's uterus.

When the movements of the uterus are studied in the body the results depend on the animal used. The response is different for example in the pregnant cat from that in the non-pregnant. In the non-pregnant cat an injection of 10 mgms. of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolium chloride causes a relaxation of the uterus and inhibition of rhythm (fig. 13). This effect persists after section of the hypogastrics. It is evident, since the isolated uterus gives a definite motor response (fig. 10), that the effect of this alkaloid upon the muscle of the uterus is overcome by an inhibitor effect generated in the cells of the post-ganglionic neurones of the sympathetic system. The dominant influence of these cells is in the non-pregnant cat inhibitor (Dale[21]).

That this is the explanation of the relaxation is probable, since when large doses of nicotine have been given, and these cells are greatly depressed, the response to the alkaloid becomes motor.

Moreover, in the pregnant cat, where the dominant sympathetic supply has become motor (Dale), the effect of this substance is to produce contraction.

Again, in the rabbit, which has a dominant motor supply in non-pregnant and pregnant conditions, the response to the alkaloid is a well-marked powerful contraction (fig. 14).

Hydrastinine gives precisely similar results. There is something peculiar with regard to the effect of cotarnine on the rabbit's uterus, for when its movements are studied *in situ* there is no contraction observable when doses as large as 25 mgms. are given intravenously (fig. 15). Yet on the excised organ it exercises a marked effect (guinea-pigs, rabbits and cats). I can offer no explanation of the anomaly.

On the uterus then there is a twofold action by these substances—

(1) direct on the plain muscle;
(2) through the sympathetic nerve cells.

The result of these two actions may be a pure motor response, as in the rabbit and pregnant cat, or an inhibitor response as in the non-pregnant and virgin cat. That this series of compounds possesses an action on the ganglion cells or the sympathetic system, supplying the uterus, is unfortunately incapable of further proof. Owing to the peripheral locality of these cells it is impossible to conduct degeneration experiments which would place the above conclusion beyond all doubt.

One feature of the action of these substances on the uterus is worthy of mention. Repeated administration of large doses for some time produces a greatly increased amplitude in the rhythm of the organ. And this persists after removal of the drug. This probably is the result of an increased excitability of the uterine muscle, induced by this series of iso-quinoline derivatives. Kurdinowski[18] was able to show increased excitability as a result of perfusing the isolated rabbit's uterus with Locke's solution containing hydrastinine in small quantity. The explanation in the one case probably holds for the other two.

Another contradiction in the pharmacology of these alkaloids is met with at this point. Kehrer found the same results as those described above with hydrastinine and cotarnine on the isolated uterus of cats and rabbits; but he describes and figures tracings which show that both these alkaloids produce contraction of the uterine muscle in the body. He states that pregnant and non-pregnant cats respond in the same manner. I am quite unable to reconcile his results with those described above, and am convinced that there must have been some source of error in his experiments which I am quite unable to indicate.

It might be thought that other cells of the sympathetic system would be affected in a similar manner. This, however, does not appear to be the case, since direct application of the new alkaloid to the superior cervical ganglion of a cat in weak and strong concentration did not cause any variation in pupil and nictitating membrane. Moreover, an excellent response could be obtained by stimulating the cervical sympathetic with a weak tetanising current (coil 23-25) both before and after application of the drug to the ganglion.

As mentioned above, the rise of blood-pressure is unchanged after full doses of ergotoxine, consequently hydrastinine and 6:7-dimethoxy

2-methyl 3:4-dihydro-iso-quinolinium chloride do not appear to have any action on the sympathetic nerve cells innervating the plain muscle of the vessels.

The conclusion is thus arrived at that this action on sympathetic nerve cells or postganglionic nerve fibre is limited to those cells or fibres innervating the uterine musculature. This strictly limited effect of this series of alkaloids on sympathetic nerve cells is somewhat unusual, and yet is the only one which will explain the facts.

On the urine flow the new alkaloid has practically no action. In one experiment a transient diminution of the urine flow was noticed after 20 mgms. of this substance had been given intravenously, accompanied by a small transient diminution in kidney volume. Fig. 16 shows the result of another experiment. The rise of blood-pressure counterbalances the vaso constriction, kidney volume is practically unaltered, and the urine flow remains unchanged. It is noteworthy that a diminution in urine flow was noticed by Falk[12] as the result of the administration of hydrastinine, while other observers could detect no difference. My experiments lead me to the conclusion that such effects as are produced by these drugs on the urine flow are so slight as to be negligible.

Excretion. It appeared probable from the fact that von Bunge[14] detected hydrastinine in urine that the path of excretion would be the same in the case of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride. 0·2 gram. of this substance was given in a gelatine capsule by mouth to a dog. The urine was collected for the next twenty-four hours, and worked up in the following manner:—After a preliminary filtration the urine was made strongly alkaline with caustic soda, and shaken out four times with chloroform. The chloroform extracts were joined together and washed with a small quantity of water. The chloroform extract was then shaken with dilute hydrochloric acid. The aqueous layer, which was of a primrose-yellow colour, was made strongly alkaline with caustic soda and the extraction with chloroform repeated. The chloroform extract was washed with water and taken to small bulk on the water bath after the addition of a few drops of hydrochloric acid. A yellow residue remained. This was crystallised from alcohol and ether and again from chloroform. The product consisted of clusters of fine primrose-yellow needles, but was contaminated by traces of amorphous brown pigment. Yield 0·065 mgms. or 32 per cent. of theoretical. The product melted at 53·5-55° C., and when mixed with its own bulk of the drug originally administered it melted at 56-57° C. The melting point

of 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride is 61-62° C. (corr.). The physical properties of the product were the same as the original product, and, moreover, the physiological action was identical; it produced a rise of blood-pressure accompanied by some slowing of the heart beat (fig. 17), and had the characteristic effect on the isolated uterus.

There can be no doubt, then, that the method of excretion of this iso-quinoline derivative is by the urine. The yield obtained was not very large, but it is very probable that if larger doses were given and the urine collected for a longer period after the administration that this would be greatly increased.

This series of iso-quinoline derivatives is without action on intestinal movements. Isolated rabbit's jejunum suspended in a Ringer bath continues its rhythmic movements, unaltered in time, extent or tonus, when any one of this series of alkaloids is added to the bath.

Bladder. The tone of the bladder is very slightly increased by 6:7-dimethoxy 1-methyl 3:4-dihydro-iso-quinolinium chloride, and this was also found to be the effect of hydrastinine. Cotarnine, on the other hand, was without effect.

Respiration. As was noticed in the earlier part of this paper, 6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride and hydrastinine and cotarnine have an effect on the respiratory centre. In large doses their depressant action on the centre is sufficient to cause cessation of respiration and death.

Slowing of the respiration without significant alteration in depth was noticed when doses of 20 mgms. of any one of the three iso-quinoline derivatives was administered intravenously and the respiratory movements recorded above the blood-pressure. Cotarnine has a slightly more marked effect than the other two.

6:7-dimethoxy 2-methyl 3:4-dihydro-iso-quinolinium chloride has practically no effect on striped muscle. A nerve-muscle preparation soaked in 1 in 1,000 solution of this alkaloid in Ringer solution for two hours responded quite well to electrical stimulation of its nerve, or of the muscle direct. This concentration is considerably above that which can occur in the body. Santesson[19] found that there was a slight diminution in sensitiveness to stimulation through the nerve when large doses of hydrastinine were given to frogs. He compares this result with those he obtained with iso-quinoline and N-methyl-iso-quinoline.[20] The methylation of the nitrogen producing a mild curare effect. The effect

is not nearly so profound as that produced by methylation of nitrogen to quaternary bases and can only be of secondary importance.

In conclusion, I desire to express my indebtedness and to thank Dr. F. Pyman for correcting the chemical formulae of this paper, and for supplying me with the new alkaloids he had prepared, also to acknowledge the kind assistance and advice of Dr. H. H. Dale throughout the work which is the foundation of this paper.

Summary

1. A review of the action of a number of iso-quinoline alkaloids is made, and their action is compared with their chemical constitution.

A law of relationship for one series is put forward.

2. The new alkaloid 2-methyl 3:4-dihydro 6:7-dimethoxy-iso-quinolinium chloride has an action very similar to that of hydrastinine.

3. The generally accepted view of the action of hydrastinine on the functions of the body is erroneous in several particulars.

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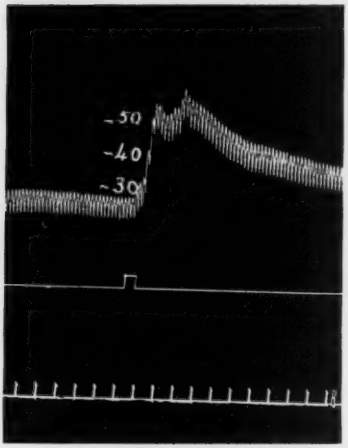


FIG. 1. Cat, decerebrate. Blood pressure, base line and signal. Time in 10″. Effect of 10 mgs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

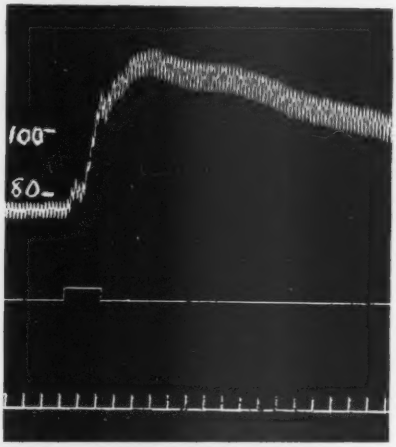


FIG. 4. Cat, decerebrate. Blood pressure, base line and signal. Time in 10″. Sufficient ergotoxine had been given previously to reverse completely the adrenine response. Effect of 20 mgrs. hydrastinine.

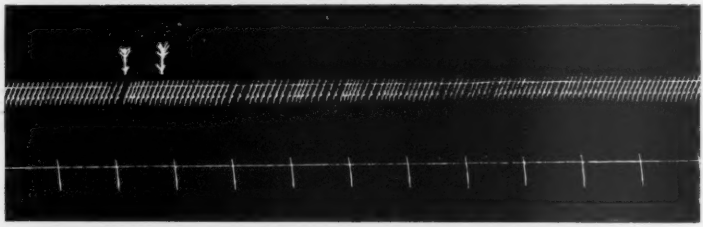


FIG. 2. Perfused rabbit's kidney. Drop record of venous outflow. Time in 10″. At arrow, 5 mgrs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride injected into the perfusion cannula.

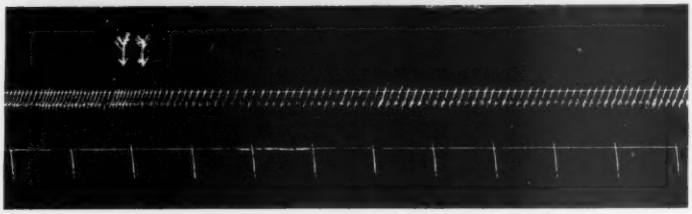


FIG. 3. Same experiment as above. Effect of 5 mgrs. hydrastinine.

FIG. 5. Venous outflow from hind limbs of rabbit. Brodie-Dixon method. Time in 10". Absence of effect on injecting 10 mgrs. cotarnine into perfusion cannula. To be read from right to left.

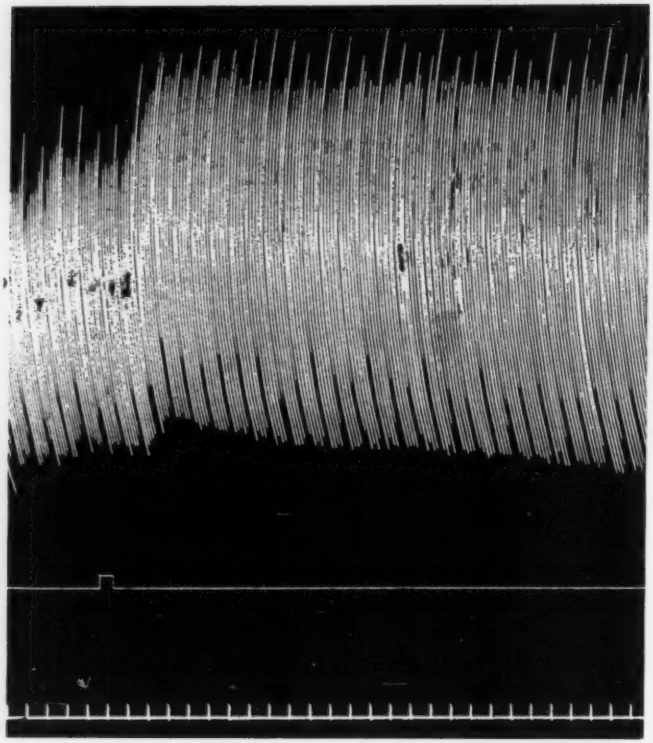


FIG. 6. Cardiometer record from cat's heart. Decerebrate cat. Effect of 50 mgrs. of 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

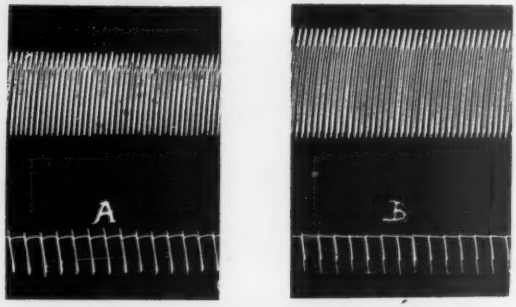


FIG. 7. Isolated rabbit's heart. Locke-Langendorf method. Time in seconds. A normal, B after 10 mgrs. hydrastinine had been injected into perfusion cannula.

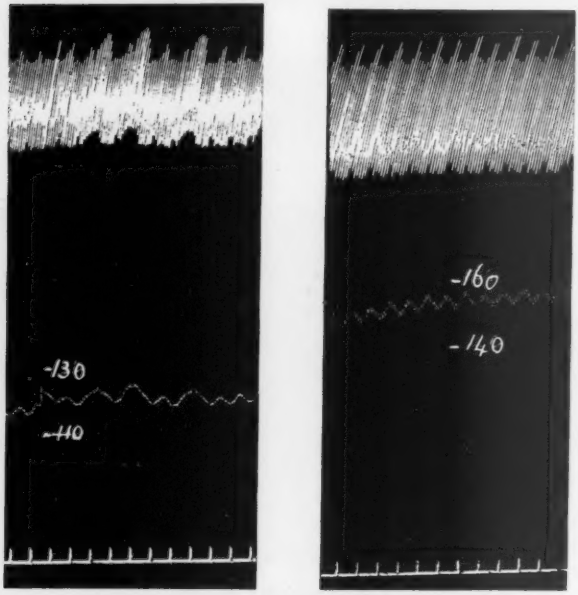
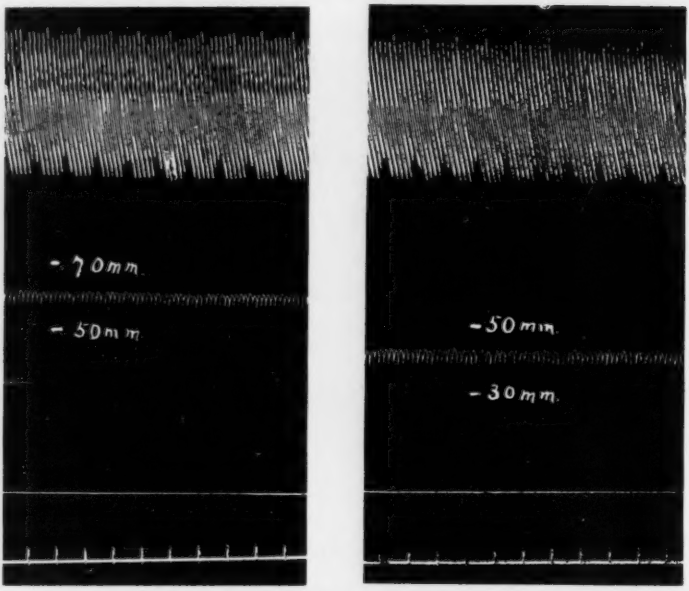


FIG. 8. Cat, paraldehyde and ether. Cardiometer blood pressure. Time in 2". A normal; B maximal effect after 20 mgrs. of hydrastinine.



A B

FIG. 9. Cat paraldehyde and ether. Cardiometer blood pressure. Time in 2″. A. Normal tracing. B. Maximal effect after 20 mgrs. cotarnine.

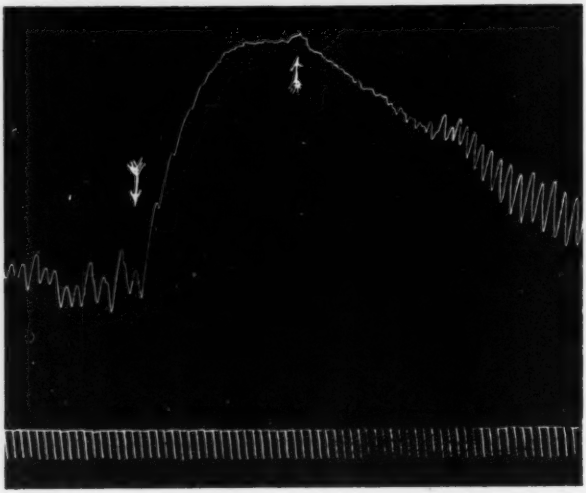


FIG. 10. Virgin cat, isolated uterus in 250 c.c. warm oxygenated Ringer suspension method. Time in 10″. At ↓ 10 mgrs. 6 : 7 dimethoxy. 2 methyl 3 : 4 dihydro-*iso*-quinolinium chloride added to bath at ↑ a change to fresh Ringer. Upstroke = contraction.

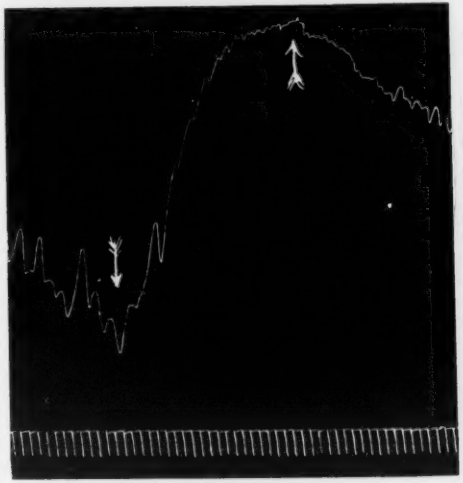


FIG. 11. From same experiment as Fig. 10. Effect of 10 mgrs. hydrastinine.

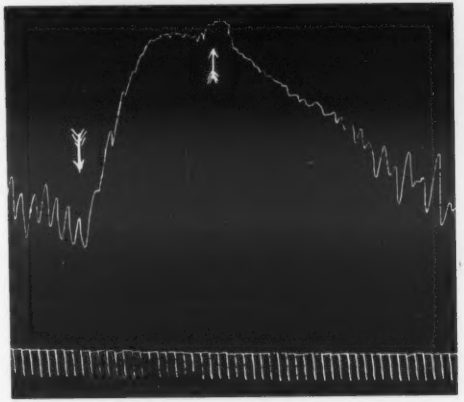


FIG. 12. From same experiment as Figs. 10 and 11. Effect of 10 mgrs. of cotarnine.

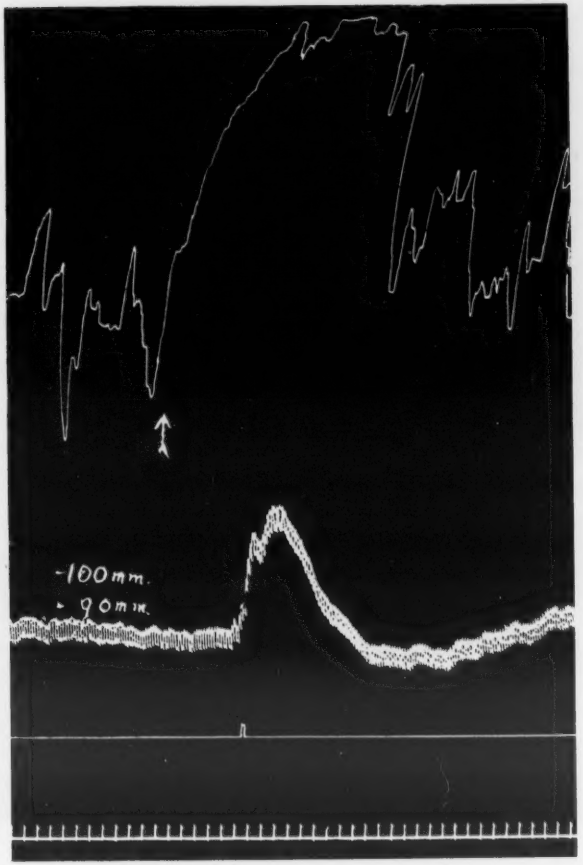


FIG. 13. Virgin cat, decerebrate. Movements of uterus, thread-pulley method. downstroke = contraction. Blood pressure base line and signal time = 10″. Effect of 20 mgrs. 6 : 7 dimethoxy 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

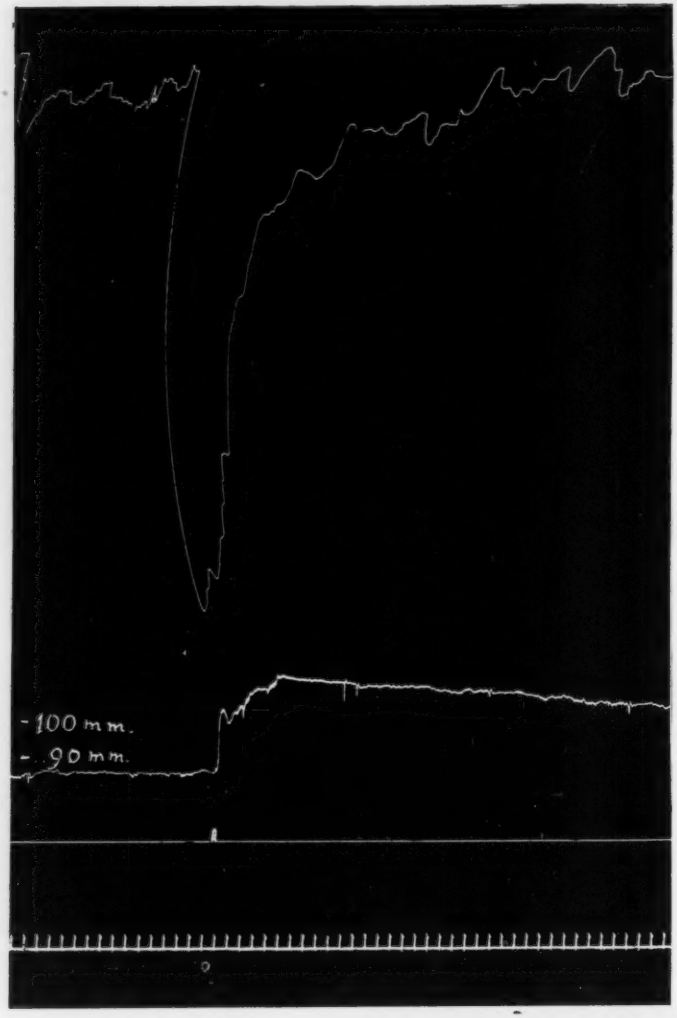


FIG. 14. Rabbit urethane. Movements of uterus, Cushny recorder, downstroke = contraction. Effect of 20 mgrs. 6 : 7 dimethoxy. 2 methyl. 3 : 4 dihydro *iso*-quinolinium chloride.

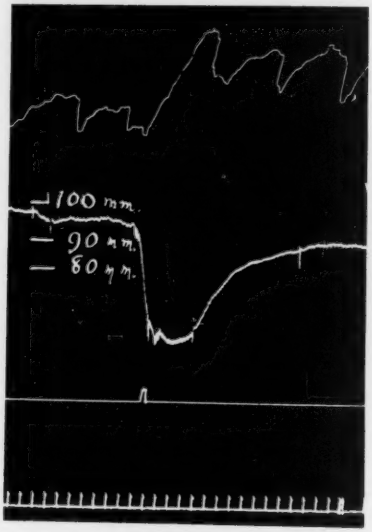


FIG. 15. Same experiment as above, effect of 20 mgrs. cotarnine.

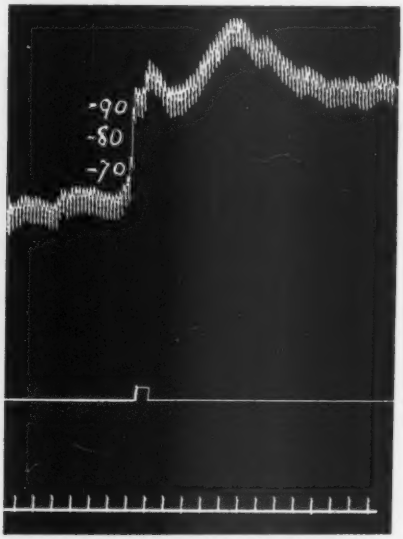


FIG. 17. Cat decerebrate. Blood pressure, base line and signal time in 10". Effect of 20 mgrs. 6 : 7 dimethoxy, 2 methyl, 3 : 4 dihydro, *iso*-quinolinium chloride recovered from the urine of a dog.

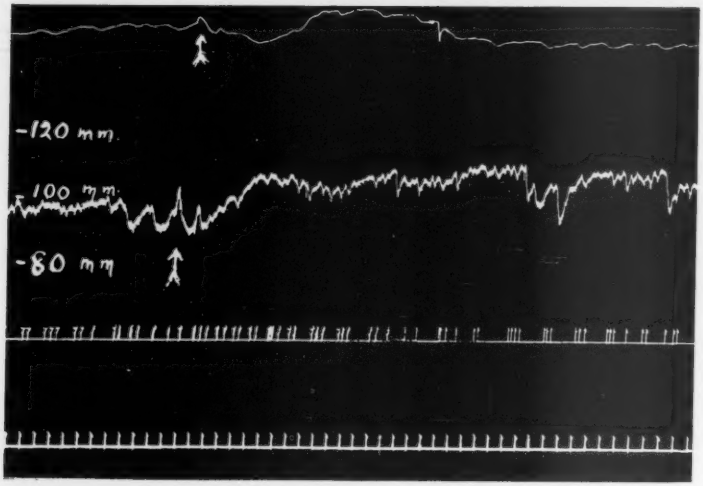


FIG. 16. Rabbit urethane, kidney volume blood pressure urine flow, time in 10", effect of 20 mgrs. 6 : 7 dimethoxy. 2 methyl 3 : 4 dihydro *iso*-quinolinium chloride.

THE ACTION OF URINARY ANTISEPTICS

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(*Received August 19th, 1910*)

The following work was undertaken with the object of investigating the action of the more important reputed urinary antiseptics, under varying conditions, especially with regard to different organisms and to the reaction of the urine.

For the purpose of the experiments the following drugs were selected:—

(1) Urotropin. This drug was chosen since it has the reputation of being the most powerful urinary antiseptic we possess.

(2) Sandal-wood oil and its derivative Santalol-salicylate, or 'Santyl,' were taken as being generally regarded as the most efficacious of the oils, balsams and resins.

(3) Salicylic acid was selected as the representative of the Coal-tar group.

The micro-organisms were chosen from groups which commonly affect the urinary tract; those which only rarely occur were not used, while two important varieties—the Tubercle Bacillus and the Gonococcus—could not be employed because of the difficulty and uncertainty of cultivating them. I therefore limited myself to the study of:—

(1) The Putrefactive Organisms.
(2) *Staphylococcus pyogenes aureus.*
(3) *Bacillus coli communis.*

The latter organism is of special interest since pure infections of the urinary tract with it are frequent.

Methods

I made use of my own urine throughout the experiments. It was tested before and after the work and found to be normal to the ordinary clinical tests, i.e., as to reaction, specific gravity, and absence of albumen, sugar and deposits. The diet, exercise and mode of life were kept as far as possible constant at the time, so that the results are

strictly comparable. The complete twenty-four hours' specimen was collected from morning to morning, and samples of that of the previous twenty-four hours used in each day's work. In the determination of acidity it was found convenient to take as an arbitrary standard a 'degree of acidity' which may be defined as the 'amount of acid in 10 c.c. of a urine of specific gravity 1020, which will exactly neutralise 1 c.c. of decinormal soda, using phenol-phthalein as the indicator.' The urine was diluted ten times with distilled water to make the colour less obvious. The first definite change of tint was taken as the end-point. The degree of acidity is then found by taking the amount, in cubic centimetres, of decinormal soda required for 10 c.c. of the given urine and dividing it by the number, beyond 1,000, in the specific gravity of the specimen, and multiplying by 20. Thus urines of different specific gravities are reduced to a common level. For example, suppose a urine of specific gravity 1015 required 2·8 c.c. of decinormal soda, then the acidity is $\frac{2{\cdot}8 \times 20}{15} = 3{\cdot}7$. By this means, as was pointed out by Joulie,[1] the acidity is given in terms of the total solids and not of the bulk of fluid.

I am, of course, aware that this direct titration of alkali does not give the true acidity of such a mixture of acid salts as the urine, nor the amount of any one acid constituent, but it is the only simple and accurate method of determining the urinary acidity,[2, 3, 4] and it gives reliable comparative results. In alkaline urine the degree of alkalinity was found by adding excess standard acid and titrating as above.

The acidity and alkalinity was varied by taking acid sodium phosphate or potassium citrate, respectively, by the mouth. In certain experiments with sandal-wood oil and with salicylic acid the acidity was increased or diminished by adding directly to the urine known amounts of NaH_2PO_4 or $NaHCO_3$. By this method specimens of different acidities can be obtained more readily than by the more laborious method of taking each drug by the mouth and sterilizing each specimen of urine; and, moreover, it was found experimentally that this method was satisfactory; that is, the same results were obtained as if the drug had been taken by the mouth.

The various drugs were taken by the mouth for periods of three to five days, and specimens of urine were not tested until the drug had been taken for at least twenty-four hours. In those experiments dealing with putrefaction the urine was incubated in chemically clean but unsterilized tubes at body temperature, and thus allowed to putrefy. In experiments

with other organisms in pure culture it was necessary to obtain sterile urine and to infect it with the organism in question. Boiling might conceivably alter the composition of the urine, and so is inadmissible; and as it is almost impossible to obtain sterile urine by the method of cleansing the meatus, &c., and passing into sterile vessels, as has been recommended, since the urine is always contaminated with the *Diplococcus urethrae*, it was sterilized by passing through a Pasteur-Chamberland filter fitted with an arrangement for collecting large quantities.

The Effect of Variations in the Acidity Alone

A number of experiments were made in which the different organisms were grown in urines of varying acidity and alkalinity. Measured by the standard already given, it was found that the average acidity of the

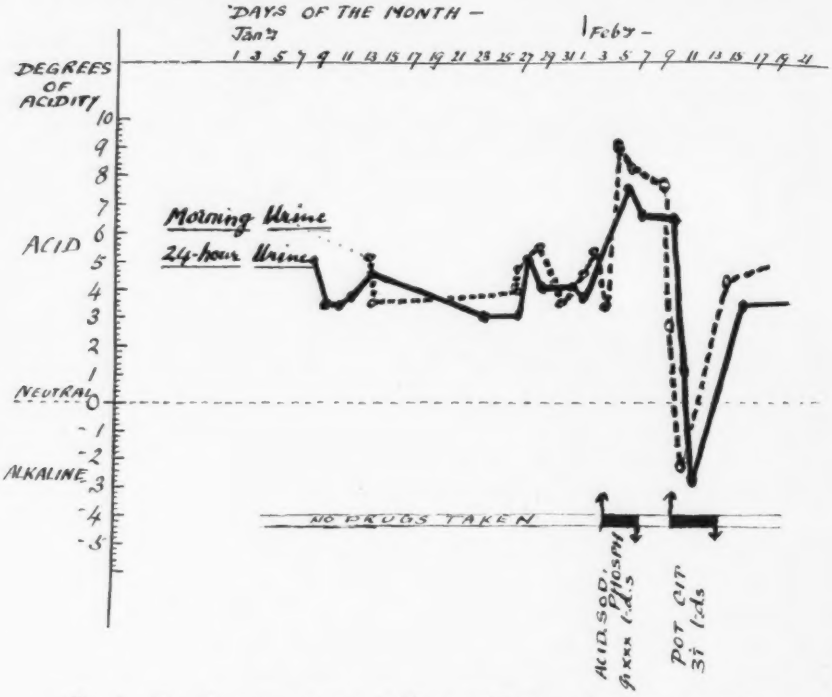


Fig. 1. The Variations in the Normal Urinary Acidity and the Effect of Drugs in altering it.

twenty-four hours' specimens of urine over a period of five weeks was 3·9, the extreme variations being between 3 and 5; the average of the morning specimens being 4·3 and the extreme variations from 3 to 5·5. By taking reasonable doses of acid sodium phosphate the acidity was raised to 8·9, while with potassium citrate the urine was readily made alkaline to the extent of 2·5 degrees. The results are shown in detail graphically in the accompanying curve (fig. 1). The results correspond closely to those of Hutchison.[2]

When a urine of average acidity is allowed to putrefy in the manner described above, it becomes alkaline in twenty-four hours, and is giving off free ammonia in two to three days. Urine which is alkaline when passed putrefies rapidly, while the putrefaction of urine of high acidity is considerably delayed.

A number of experiments were made with a view to determining the effect of the degree of acidity upon putrefaction (fig. 2).

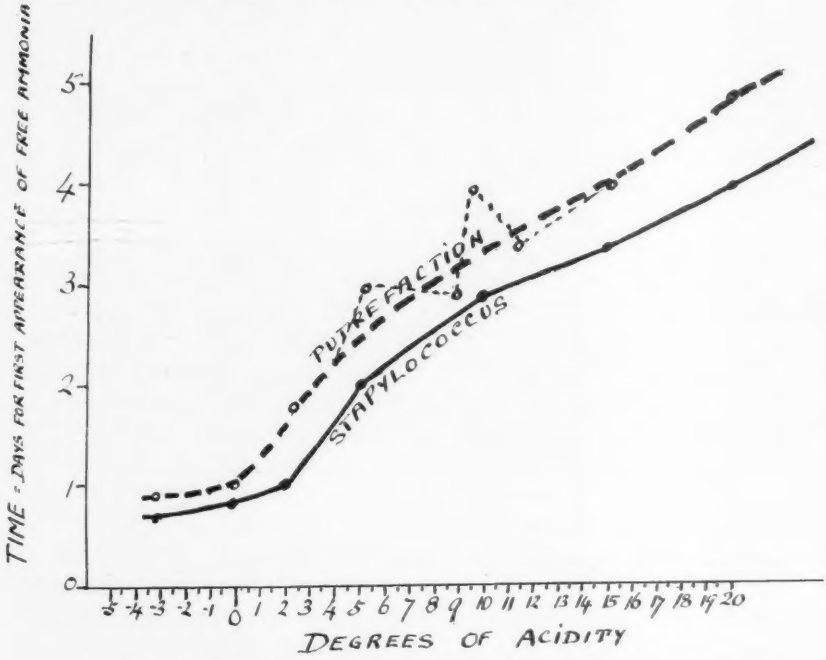

TIME = DAYS FOR FIRST APPEARANCE OF FREE AMMONIA
0
1
2
3
4
5
PUTREFACTION
STAPHYLOCOCCUS
-5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DEGREES OF ACIDITY

FIG. 2. THE EFFECT OF VARIATIONS IN THE ACIDITY UPON THE RATE OF ALKALINE FERMENTATION.

Experiment I.—Specimens of urine incubated at body temperature.

(*a*) 24-hours' urine. Jan. 14-15.
Sp. Gr. 1016. 2·8 c.c. of decinormal soda required for 10 c.c. Acidity = 3·5.
Result—Alkaline in less than 24 hours, ammoniacal in 36 hours.

(*b*) 24 hours' specimen, February 11-12.
Acidity = − 2·5 (alkaline).
Result—Ammoniacal in 24 hours.

(*c*) Morning specimen, February 3-4.
Acidity = 8·9.
Alkaline in 2 days, and ammoniacal in three.

(*d*) Specimen to which acid sodium phosphate had been added, artificially raising the acidity to 20.
Result—Alkaline in 4 days, and ammoniacal in 5 days.

To stop putrefaction altogether the acidity must be raised to 80 or 90, i.e., seven or eight times as high as it is possible for it to be raised by giving drugs by the mouth.

In the experiments with *Staphylococcus pyogenes aureus*, tubes of filtered urine were taken, shown to be sterile by incubating for twenty-four hours without change, and sown with *Staphylococcus* from a young agar culture. The tubes were then incubated at body temperature. In this way the *Staphylococcus* is found to grow very readily in urine and is seen as a deposit of minute whitish granules which tend to adhere to the sides of the tube. It breaks up urea, giving rise to ammoniacal fermentation, but the characteristic foul smell of advanced putrefaction is generally absent. Its growth is favoured by alkalinity, and is delayed by acidity in the same manner as is putrefaction. The time of the first appearance of free ammonia, as in the case of putrefaction, was taken as the gauge of the rate of growth, and similar experiments to the putrefactive ones were performed. The results are illustrated in fig. 2. The only differences of importance are that the *Staphylococcus* is a little more active and the experiments more constant than those with the mixed organisms of putrefaction.

The *Bacillus coli* was grown in sterile urine in the same way as the *Staphylococcus*. It grows very readily and appears as a fine cloudy deposit which renders the urine uniformly turbid. On shaking or stirring, peculiar glistening swirls are seen, and these are not present in cloudy putrefying urine, nor have I observed the appearance in connection with any deposit or substance in urine except with bacteria of the Coli group and with *Bacillus typhosus*. The *B. coli* is sometimes seen in urine joined end to end, forming a long spirillum-like structure

which in a hanging drop worms its way among the normal motile bacilli. Sown back to agar the bacillus assumes its normal form, and these curious forms appear to occur more frequently in old growths in urine. In urine made artificially many times more acid or more alkaline than occurs in the body, the *B. coli* will grow; and, despite what is said to the contrary, no marked differences are observed in its rate of growth whether the urine be acid or alkaline. The *B. coli* does not cause alkaline fermentation, and in consequence it is impossible to obtain any such simple indication of its rate of growth as with the other organisms. I therefore made the simple observations that either (1) the organism grew readily, (2) grew with difficulty, or (3) grew not at all. Until limits of acidity and alkalinity beyond those which occur in the body were reached, it was impossible to appreciate any differences in the rate of growth. It is possible that the statement often made that its growth is inhibited by alkaline urine, is due to an erroneous assumption from the fact that the bacillus, when growing alone, tends to occur in acid urine only, such being the case because urine is normally acid, and *B. coli* has no tendency, as have most of the other bacteria which grow in it, to make it alkaline.

The Effect of Drugs

(1) *Urotropin*

Hexamethylenetetramine, commonly known by the trade name of urotropin, was discovered by Butlerow in 1860 as a chemical substance, and introduced as a drug in 1894 by Nicolaier. It was first suggested as a solvent for uric acid in the same way as was piperazine. The use of both these drugs as solvents of uric acid in the body is now discredited, but the action of urotropin as a urinary antiseptic is established. The fact that urotropin is synthesised from formaldehyde, and readily yields that substance on boiling with acids, suggested that its action was due to the liberation of formaldehyde in the urine. Nicolaier[5] in his early papers on the subject showed that the disinfecting power of urotropin in the urine varied a good deal with temperature and other conditions, and he expressed the view that the action of the drug was due to the formation of formaldehyde. Caspar[6] demonstrated formaldehyde in the urine of certain patients taking urotropin. Citron[7] considered that the formaldehyde was present as a loose soda compound. Cotyl and Salus[8] came to the conclusion that the antiseptic action was

due to urotropin itself, and not to formaldehyde. Cammidge[9] failed to find formaldehyde in the urine, and concluded that urotropin does not act by its formation. An excellent summary of the whole subject to the year 1905 will be found in a series of papers by Guiard.[10]

Experiments with Urotropin.—

(1) Bacteriological Experiments as to the antiseptic power of the drug.
Samples of 24 hours' specimens of urine, obtained as follows, were used :—

A. Urotropin, 10 grs. t.d.s. taken.
Acidity + 1·5.

B. Urotropin, 10 grs. with acid sodium phosphate, 30 grs. t.d.s.
Acidity + 7·0.

C. Urotropin, 10 grs. with potassium citrate, 60 grs. t.d.s.
Acidity — 1·5 (alkaline).

From these three specimens some samples were allowed to putrefy, while others were sterilized by the filter and sown with the organisms in pure culture as in the previous experiments on acidity.

Table of Results

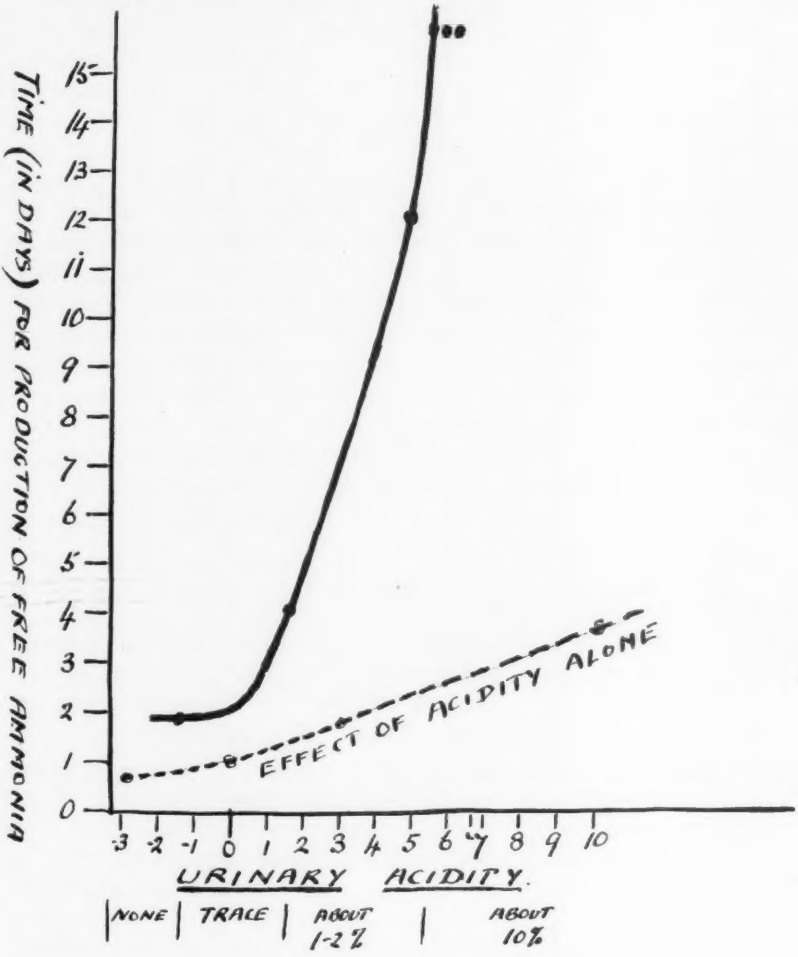
In all cases the urine was incubated at body temperature.

Urine of	Acidity	Putrefies in	Staphylococcus renders Ammoniacal in	B. coli.
C.	Acid — 1·5 (alkaline)	2 days	3 days	Grows freely.
A.	Acid + 1·5	4 „	4 „	Grows not freely.
	Acid + 5·0	12 „	10 „	No growth in 14 days
B.	Acid + 7·0	No change after 14 days' incubation in any case.		

Further experiments with urines of intermediate degrees of acidity were done, the acidity being varied by adding acid sodium phosphate or sodium bicarbonate directly in the manner already described. In all cases the same dose of urotropin was taken by the mouth. The results are shown, with those tabulated, in the subjoined curve (fig. 3).

These experiments demonstrate that the antiseptic power of urotropin in alkaline or neutral urine is almost nil, that this power rises rapidly as the acidity increases, and when the latter reaches about 4 (i.e. slightly above the normal) it becomes absolute and the urine remains indefinitely sterile. Furthermore, no differences of any importance were here observed in the cases of the different organisms: all were affected in much the same way. These experiments can only be explained by the

assumption that in the urines of higher acidity there is present an antiseptic of considerable power which does not exist in the urines which are alkaline or of low acidity.



Probable percentage of Urotropin which has yielded Formaldehyde.

FIG. 3. TO SHOW THE EFFECT OF ACIDITY UPON UROTROPIN. (AGAINST PUTREFACTION.)

The degree of antiseptic power of these acid urines was to some extent ascertained as follows:—It was found that when urotropin urine of acidity 7 (specimen B) was added in varying proportions to normal urine of acidity 3·9, one part of this urotropin urine with nine parts of the normal urine was sterile when incubated. Now in these experiments 30 grains of urotropin was taken in the twenty-four hours, and about 50 ozs. of urine was secreted during that time. Urotropin is shown by the bromine water test to appear in the urine about twenty minutes after its administration, and to persist for about twenty-four hours after this has ceased. Not all, but probably the greater part, of the 30 grains is therefore contained in the 50 ozs. of urine, so that the strength of the substance secreted in the urine will be about 1 in 1,000 or less, and this further diluted to the extent of 1 in 10 is, as we have seen, sufficient to prevent putrefaction. I found that if carbolic acid be added to urine to stop putrefaction, about 1 in 800 is required for the purpose, so that the antiseptic substance in acid urotropin urine must be at least ten times as powerful as carbolic acid. Moreover, Mosso and Paleotti[11] have shown that, if formaldehyde be added directly to urine, it stops putrefaction when of a strength of 1 in 10,000, and has a definite antiseptic effect when present 1 in 50,000, so that it is obvious that the substance present in acid urotropin urine corresponds closely in antiseptic power with formaldehyde.

It remains to demonstrate the presence of formaldehyde in urotropin urine, and, if possible, to determine the conditions of its formation. It is upon a failure to do this that the workers, who have come to the conclusion that urotropin does not act by the formation of formaldehyde, have based their views. Cammidge[9] showed that 5 per cent. of urotropin in neutral solution was required to exert the same antiseptic power as 0·08 per cent. of formaldehyde. It is obvious which of these figures corresponds most nearly to the maximum possible strength of the substance present in a urotropin urine with antiseptic power, yet Cammidge concluded from a failure to detect it chemically that no formaldehyde was present. He used as tests for formaldehyde the reduction of Fehling's solution, the resorcin test, and Schiff's rosanilin reaction. I repeated some of his experiments with results corresponding to his. He does not appear to have realised in how small an amount the formaldehyde would be, if present, for he mentions the fact that urotropin urine never smells of formaldehyde as evidence against its presence. A solution, of 1 in 10,000, which would exert quite a powerful

antiseptic action, as we have seen, would, of course, have not the slightest smell. Moreover, none of Cammidge's tests, if tried upon a 1 in 10,000 solution of formaldehyde, are positive. There is, however, an extremely delicate test which will readily detect 1 in 200,000 of formaldehyde, or even less. This is the phloroglucin test. A 1 per cent. solution of phloroglucin with 25 per cent. of alcohol is made with distilled water, and a little strong caustic soda solution added. A few drops of this gives a bright cherry-red colour with solutions of formaldehyde. In testing for small amounts the solution should be added slowly, and the colour appears in a few seconds and fades after a time (probably by reason of the combination of the formaldehyde and the soda). The test solution must be fresh. With this test I have never failed to detect formaldehyde in acid urotropin urine. A distinct red colour is produced on adding the test fluid directly to the urine which is not produced in normal urine or in alkaline urine containing urotropin. The demonstration may be made more obvious by distilling the urine 'in vacuo' at body temperature and testing the distillate, when a bright cherry-red colour is produced. Boiling is of course inadmissible, since formaldehyde might then be produced by the heat.

Experiments to elucidate the conditions under which formaldehyde is formed in urotropin urine:—

Broth Culture Experiments with Urotropin.

Broth cultures to which drugs had been added as described were infected with the Staphylococcus and incubated for 24 hours.

Drugs added to culture	Result
(1) Urotropin 1 in 1000 with trace of NaH_2PO_4	No growth
(2) Urotropin 1 in 300 with trace of NaH_2PO_4	No growth.
(3) Urotropin 1 in 100 alone	Grew slightly.
(4) Urotropin 1 in 20 alone	No growth.
(5) Urotropin 1 in 20 with trace of $NaHCO_3$	Grew slightly.

These experiments demonstrate that outside the body the addition of a trace of an acid salt to urotropin will enormously increase its antiseptic power. They also show that in neutral solution a strength of 1 in 20 will inhibit growth, but that this property is destroyed by the addition of alkali; an explanation of this fact is given later.

Chemical Experiments. By means of the phloroglucin reaction not only can very small amounts of formaldehyde be detected, but, in addition, from the depth of colour a rough indication of the strength of the solution can be obtained. For this purpose the test is applied and

the colour matched by adding the same amount of phloroglucin solution under identical conditions to solutions of formaldehyde of different known strengths. When the colour is identical, the strength of the formaldehyde may be assumed to be nearly so. In this way a rough quantitative estimate is obtained.

Experiments. A solution of urotropin in distilled water of strength 1 in 1,000, i.e., about the strength in which it occurs in urine, was made up, and samples of it were incubated at body temperature for twenty-four hours.

(*a*) In neutral solution it was found that the slightest trace of formaldehyde was liberated, probably about 1 in 200,000.

(*b*) In solution with a small amount of acid sodium phosphate, or a trace of a mineral acid, a strong reaction was given, the colour indicating a strength of about 1 in 10,000.

(*c*) In solution with a small amount of sodium bicarbonate or other alkali no reaction is given, even after prolonged incubation. This, of course, explains the differences in the behaviour of tubes 4 and 5 in the last described broth-culture experiments.

(*d*) Lastly, if a solution is acidified, incubated, and gives the reaction indicating about 1 in 10,000 formaldehyde, and to it is then added sufficient sodium bicarbonate to make it alkaline, together with a slight trace of ammonia, after twenty-four hours' incubation it fails to show the least trace of formaldehyde, all the urotropin has been reconstituted.

It will be seen that these chemical experiments are in every respect consistent with those in which the actual effects of urotropin as a urinary antiseptic were investigated, provided that it be accepted that its powers are due to formaldehyde. Urotropin in solution is a comparatively unstable substance. In acid solution it tends to disintegrate into formaldehyde and ammonium compounds. In alkaline solution the reaction tends the other way. Various factors—the dilution, the time allowed, and above all the temperature—will affect these reactions, but from a pharmacological point of view one is only concerned with these factors as they occur in the body when urotropin is being given as a drug, namely, with a dilution of 1 in 1,000 or less, and with effects taking place during a number of hours at a temperature of about 37° C. If these conditions are observed, it is found that urotropin in neutral

solution disintegrates, yielding formaldehyde to a very small extent, namely, about 0·05 per cent. to 0·1 per cent. In alkaline solution no dissociation occurs. In acid solutions the percentage of urotropin which breaks up increases considerably, in a degree related to the acidity, so that with an acidity corresponding to that of acid urine about 10 per cent. is split up into formaldehyde.

It has been shown that these processes occur in urine when urotropin has been taken, and that the antiseptic power of the drug runs parallel with, and can only be explained by, the amount of formaldehyde present. So that the object in giving urotropin as a urinary antiseptic is to exhibit formaldehyde in the urine, and to keep this in view constitutes, I believe, the key to the correct use of the drug.

(2) *Sandal-wood Oil*

Sandal-wood oil has largely superseded copaiba and cubebs, and is generally reputed to be the most efficient of the essential oils. It contains two substances allied to the sequiterpenes, santalol and santalal. In the last few years these substances, or combinations of them, have been put upon the market under the names of gonosan, santyl, etc. Santyl, which is santalol-salicylate, is highly praised from a clinical standpoint,[12] and in consequence some experiments were done with it to compare its efficiency with that of the crude oil.

Sandal-wood oil, like most other essential oils, is excreted in the urine partly unchanged and partly as a compound of glycuronic acid. Its reputation as a urinary antiseptic is not great, though it is acknowledged to be of value in the treatment of gonorrhoea. It would seem that here it must act as an antiseptic, though some writers regard its action rather as an 'astringent' to the mucous membrane, because in cases of gonorrhoea which are benefited by sandal-wood oil, other and more powerful antiseptics are said to be of little avail. This astringent speculation is nonsense; none of the essential oils exhibit astringent properties. Urotropin has not been given a full trial; Leedham-Green[13] found it useful in certain stages of the complaint.

Experiments with sandal-wood oil. The sandal-wood oil was taken in gelatine capsules in doses of 20 m. three times a day, and specimens of the resulting urine were used. The acidity of the specimens was varied by the method of direct addition (v. s.).

(1) Putrefaction :—

Acidity	Time, in days, for first appearance of free ammonia
− 2 (alkaline)	1 day
+ 3·5	4 days
+ 10	7 days

Some cloudiness in 24 hours in all these tubes.

(2) Growth of *B. coli* in sterilised sandal-wood oil urine : —

Acidity	Result
− 2 (alkaline)	Growing freely in 24 hours.
+ 3·5	
+ 10	
+ 15	Growing freely in 48 hours.

(3) Growth of Staphylococcus in sterilised sandal-wood oil urine :—

Acidity	Time for first appearance of free ammonia
− 2 alkaline)	3 days
+ 3·5	9 days
+ 10	14 days
+ 15	Growing but not alkaline in 14 days

It will be seen that the action of sandal-wood oil against putrefaction is very feeble. It about doubles the time the urine takes to putrefy. In the case of the *Bacillus coli* there is practically no antiseptic action at all, this organism growing quite well in sandal-wood oil urine. With the *Staphylococcus*, however, the case is entirely different; here there is quite a powerful antiseptic effect. In alkaline urine sandal-wood oil is far more potent against the *Staphylococcus* than is urotropin, while though in urines of higher acidity it does not entirely prevent the growth of the organism as does urotropin, it has yet a marked and powerful effect.

This selective action of the drug upon a particular organism was so unexpected that to exclude the possibility of error all these experiments with the *Staphylococcus* were repeated, using controls of normal urine of corresponding acidities, but similar results were obtained and there seems no question of the accuracy of the observations.

It was noticed that in the sandal-wood oil urine, which was undergoing putrefaction, while there was a delay in the appearance of free ammonia the cloudiness of commencing putrefaction appeared fully as early as it does in normal urine. It may be suggested that this is due to a restraining influence of the drug on the putrefactive cocci rather than the bacilli, if, as seems likely, we assume that the cocci are the chief

agents in breaking up urea. Moreover, the fact of the value of sandal-wood oil against the gonococcus would be explained if we assume this selective action to extend to cocci generally. However this may be, there seems to be no doubt that sandal-wood oil has a considerable degree of power in preventing the growth of *Staphylococcus*, while towards the other organisms it is almost inert.

Experiments with Santalol Salicylate ('Santyl'). To obtain some idea of the comparative values of sandal-wood oil, and of its derivative santalol salicylate ('santyl'), some specimens of urine after taking the latter drug were allowed to putrefy and the results compared with those obtained with the crude oil.

Experiments.—

Doses of santyl of 30 m. t.d.s.	24 hours' urine used.
Acidity.	Time for first appearance of free ammonia
— 2 (alkaline)	2 days.
+ 2 (acid)	3 days
+ 5 (acid)	5 days.
+ 10 (acid)	8 days.

These results are slightly better than those with the crude oil, but since the dose was nearly double that of the latter it must be concluded that santalol salicylate has a rather feebler action than has sandal-wood oil; this can, however, be made up for by the fact that the drug can be given in larger doses, and it is less unpleasant to take.

(3) *Salicylic Acid*

Salicylic acid was chosen as the representative of those coal-tar products which have a urinary antiseptic action. Of these, salol—phenyl salicylate—is reputed to be the most powerful, but since it has a double action, due to its two constituents, it seemed not to be so suitable for experimental purposes.

Salicylic acid is excreted in the urine as salicyluric acid and as sodium salicylate. In an acid urine some of this sodium salicylate is split up, yielding the free acid. As a pure antiseptic it probably does not matter much in what form salicylates are taken, but the acid itself has the power of increasing the urinary acidity to some extent, and it was among many drugs used for this purpose before the introduction of acid sodium phosphate. With a view to getting some idea of this secondary action, I took salicylic acid, instead of any of its derivatives.

Experiments with Salicylic Acid. Salicylic acid in doses of 20 grains three times daily. Twenty-four hours specimens of urine used. No appreciable increase in the acidity of the twenty-four hours urine resulted. The twenty-four hours specimen with which the pure culture experiments were made had an acidity of 3·5, i.e., slightly below the average. Hutchison,[2] who investigated this point, found that the acidity was increased. Possibly, had the drug been taken for a longer period, the average acidities would have shown some increase, but my result shows that this action is very feeble and quite insignificant.

For investigating the antiseptic action experiments were done of the same kind as with the other substances.

Putrefaction.—

Acidity		Time taken to become alkaline		Time taken to become ammoniacal
— 3·5 (alkaline)	...	1-2 days	...	2 days
+ 3·5	...	4 days	...	5 days
+ 10	...	8 days	...	10 days

Some specimens of higher acidities were clear at the end of ten days.

Experiments with the Staphylococcus.—

Acidity		Time taken to become alkaline		Time taken to become ammoniacal
— 2 (alkaline)	...	2 days	...	3 days
+ 3·5	...	3 days	...	4 days
+ 10	...	Not alkaline at the end of 10 days.		

Experiments with the Bacillus Coli. In urines of all acidities up to 10 the bacillus was growing well in twenty-four hours, in the urines of higher acidity it was forty-eight hours before any growth was apparent, and the organism was not growing well then.

It will be noticed that the antiseptic power of salicylic acid in the urines of high acidity is somewhat greater than can be accounted for by the increase in the acidity alone; this may be due to an alteration of the proportions between salicylic acid and salicyluric acid.

Conclusions

1. Using a defined arbitrary standard, the average acidity of normal urine was about 4. With the largest doses of acid sodium phosphate (NaH_2PO_4) which can be conveniently tolerated, this was raised to 9. The urine was readily made alkaline with potassium citrate.

2. Putrefaction will occur readily in, and the *Staphylococcus* or *Bacillus coli* will grow easily in, urines of all degrees of acidity and alkalinity which can be produced in the body. The acidity exercises a restraining influence upon the putrefaction, or the growth of *Staphylococcus*, which is proportional to its degree; thus, between the limits of alkalinity on the one hand, and the highest degree of acidity which can be produced on the other, the time of ammoniacal fermentation is increased about five-fold. The *B. coli* was found to grow about equally well in urines of all degrees of acidity and alkalinity within the above limits. It grows as well in alkaline as in acid urine.

3. *Urotropin* acts as a urinary antiseptic by the formation of formaldehyde. In an alkaline urine no formaldehyde is formed, and in consequence the antiseptic action is nil. In neutral or faintly acid urines a small amount, probably not exceeding 1 per cent., is split up, and the action is feeble. The percentage of formaldehyde, and with it the antiseptic power, increases with the acidity until with an acidity of 4-5 (i.e., slightly above normal) no organism will grow in the urine, which therefore remains indefinitely sterile; at this point there is reason to believe that about 10 per cent. of the urotropin has been converted into formaldehyde. The process of conversion into formaldehyde is a simple chemical one, which occurs in all solutions of urotropin under conditions of dilution, temperature, acidity, and time which are identical with those obtaining in the body. It was found that urotropin acted equally well with the different organisms used.

4. The antiseptic action of *sandal-wood oil* in the urine was found to be quite feeble against putrefactive organisms or the *B. coli*, but it showed a marked selective action upon *Staphylococcus*, against which it has quite a specific antiseptic power.

5. The power of *Salicylic Acid* to increase the urinary acidity was very slight. As an antiseptic it has quite a definite action, though not a very powerful one. Against the putrefactive organisms or the *B. coli* it is much more efficient than sandal-wood oil; it has, however, no selective action upon any organism, and in consequence is inferior to sandal-wood oil for the *Staphylococcus*.

A diagram (fig. 4) is given in which the comparative values of the drugs are represented. It will be seen that urotropin acting in a urine of reasonably high acidity is for all purposes far and away the most powerful. If the urine is alkaline, neutral, or only faintly acid,

salicylic acid is the most efficient of these three substances against either putrefactive organisms or the *B. coli*, while sandal-wood oil is the more efficient for the *Staphylococcus*. The action of neither of these drugs, however, can be compared to that of urotropin in an acid urine.

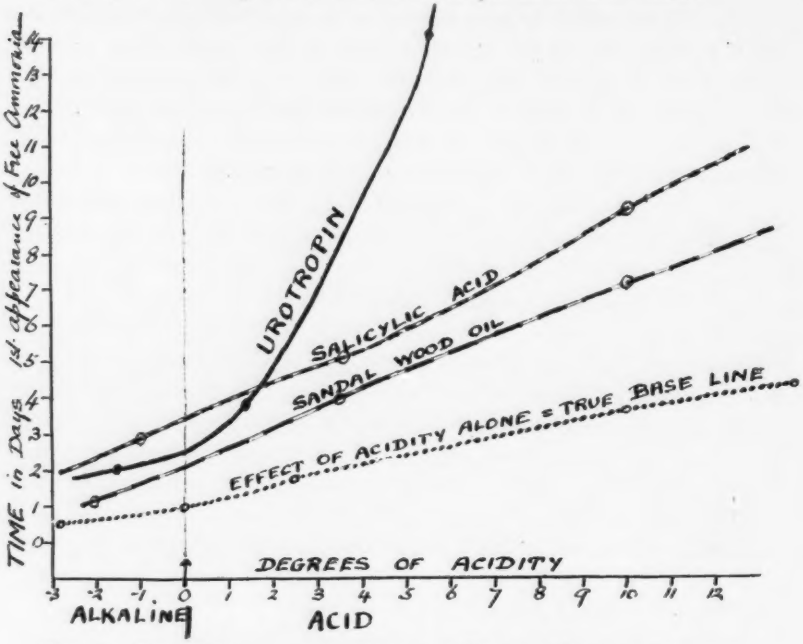


FIG. 4. THE EFFECT OF THE DEGREE OF URINARY ACIDITY UPON THE ANTISEPTIC POWER OF THE DIFFERENT DRUGS AGAINST PUTREFACTION.

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THE LAW REGULATING HAEMOLYSIS OF ERYTHROCYTES IN HYPOSMOTIC SALINE SOLUTION OR DISTILLED WATER

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In studying the haemoglobin-value of the resistant erythrocytes[1] when blood is treated with hyposmotic saline or distilled water, I have obtained results which lead to the conclusion that the amount of haemoglobin in a suspension of erythrocytes that can be dissolved in a definite volume of hyposmotic saline solution or distilled water is proportional to the total amount of haemoglobin present in the suspended corpuscles. The suspension of the erythrocytes was obtained by taking a volume of blood, which was allowed to clot after being quickly centrifuged. The clot was separated and the erythrocytes washed three or four times with 0·85 per cent. solution of sodium chloride, and a suspension of the erythrocytes obtained. One volume of this was treated with two volumes of distilled water, or N/50 sodium chloride solution. The amount of haemoglobin dissolved was then estimated, and compared with the total amount of haemoglobin present in the suspended corpuscles. The blood of various animals was thus tested and the results compared.

In the following tables, t denotes the total amount of haemoglobin in a suspension of erythrocytes, d the amount of dissolved haemoglobin in any particular suspension of erythrocytes, and $p = \frac{d}{t}$

TABLE I (*a*)

Normal Human Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of N/50 NaCl solution)—

1. $t = 110$; $d = 62$. $p \times 10^2 = 56{\cdot}3$
2. $t = 100$; $d = 58$. $p \times 10^2 = 58{\cdot}00$
3. $t = 90$; $d = 50$. $p \times 10^2 = 55{\cdot}55$
4. $t = 77$; $d = 44$. $p \times 10^2 = 57{\cdot}14$

TABLE I (*b*)

Normal Human Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 106$. $p \times 10^2 = 70{\cdot}66$
2. $t = 118$; $d = 90$. $p \times 10^2 = 76{\cdot}27$
3. $t = 50$; $d = 35$. $p \times 10^2 = 70{\cdot}00$

TABLE II

Fowl's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 105$; $d = 88$. $p \times 10^2 = 83{\cdot}80$
2. $t = 96$; $d = 78$. $p \times 10^2 = 81{\cdot}25$
3. $t = 90$; $d = 80$. $p \times 10^2 = 88{\cdot}88$
4. $t = 84$; $d = 68$. $p \times 10^2 = 80{\cdot}95$
5. $t = 42$; $d = 34$. $p \times 10^2 = 80{\cdot}95$

TABLE III (*a*)

Sheep's Blood (1 part of erythrocytes suspended in the serum of the sheep + 2 parts of distilled water)—

1. $t = 120$; $d = 120$. $p \times 10^2 = 100{\cdot}00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100{\cdot}00$

TABLE III (*b*)

Sheep's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 150$. $p \times 10^2 = 100{\cdot}00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100{\cdot}00$

TABLE IV (*a*)

Frog's Blood (1 part of erythrocytes suspended in the serum of the frog + 2 parts of N/50 NaCl)—

1. $t = 46$; $d = 10$. $p \times 10^2 = 20{\cdot}87$
2. $t = 28$; $d = 8$. $p \times 10^2 = 28{\cdot}57$

TABLE IV (*b*)

Frog's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 90$; $d = 10$. $p \times 10^2 = 11{\cdot}11$
2. $t = 70$; $d = 8$. $p \times 10^2 = 11{\cdot}42$

TABLE V

Cat's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 122$; $d = 106$. $p \times 10^2 = 86{\cdot}88$
2. $t = 62$; $d = 55$. $p \times 10^2 = 88{\cdot}71$

TABLE VI

Value of $p \times 10^2$ in the blood of a number of healthy students, the blood being treated with 2 parts of N/50 NaCl (deduced from the author's paper in the *Bio-Chemical Journal*, Vol. IV, Nos. 5, 6, and 7)—

1	2	3	4	5	6
58·4	58·4	60·9	64·3	56·4	56·3

From the above observations the following laws can be deduced:—

1. The amount of haemoglobin dissolved in a given volume of hyposmotic sodium chloride solution or distilled water is proportional to

the amount of haemoglobin in the erythrocytes presented for solution in the form of a suspension.

2. The ratio between these above two factors varies in different animals, being the smallest in our table in the case of the frog, and greatest in the case of the sheep. (In the case of the rabbit it is also very high.)

3. It is fairly constant in healthy individuals, but varies widely in disease.[1]

From the above it will be seen that the same law that regulates the solution of globulin in solution of neutral salts also regulates the solution of haemoglobin contained within erythrocytes. In other words, the latter follows the law enunciated by Mellanby and Hardy.[2] This fact probably throws some light as to how haemoglobin exists inside erythrocytes. Each individual erythrocyte allows a certain quantity of water to enter into its substance by process of osmosis when blood is treated with a hyposmotic sodium chloride solution or distilled water. If, now, we consider that the haemoglobin exists in the form of a suspension inside erythrocytes, it follows, from the law of Mellanby and Hardy, that the amount of haemoglobin that will be dissolved by water entering into the substance of the erythrocytes is proportional to the amount of haemoglobin in each individual corpuscle. Accordingly, the amount of dissolved haemoglobin that will pass out of the corpuscles is proportional to the amount of haemoglobin inside them. It follows, therefore, that the amount of haemoglobin dissolved in a suspension of erythrocytes when the latter is treated with distilled water or hyposmotic saline, is proportional to the total amount of haemoglobin present in them. We can, therefore, conclude that haemoglobin as it exists inside erythrocytes is in a state of suspension.

REFERENCES

1. *Bio-Chemical Journal*, Vol. IV, p. 280, 1909.

2. *Journal of Physiology*, Vol. XXXIII, 1905; also referred to in the *Proceedings of the Royal Society*, Series B, Vol. LXXIX, 1907.

SOME EXPERIMENTS ON THE SOLUBILITY OF GASES IN OX BLOOD AND OX SERUM

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(Received October 10th, 1910)

The question of the solubility or absorption of gases, more especially oxygen and carbon dioxide, in blood is one which has claimed the attention of many physiologists.[1] Until a few years ago the purely chemical explanation of absorption was practically undisputed; the absorption of oxygen by blood being attributed chiefly to the formation of a compound with the haemoglobin, and of carbon dioxide to the reputed alkalinity of blood and the formation of carbonates and bicarbonates. Recently, however, the remarkable surface actions of colloidal substances have been increasingly investigated by physical chemists, and it was suggested by Findlay and Harby,[2] and by Wo. Ostwald,[3] that the absorption was due, for the most part, to the surface action of the colloids in blood. This view was fully discussed by Wo. Ostwald[4] on the basis of the experimental material then available.

As scarcely anything was then known regarding the influence of colloids on the solubility of gases, we investigated the influence of a considerable number of colloids and fine suspensions on the solubility in water of carbon dioxide and nitrous oxide[5] under a range of pressures from 760 mm. to about 1,400 mm. of mercury. The results obtained were much more complex than the simple adsorption theory led us to expect; but one of the main conclusions reached was that surface adsorption is not sufficient in itself to produce a marked increase in the solubility of a gas in water at atmospheric pressure. On the contrary,

1. See, for example, Bohr's monograph *Blutgase und respiratorischer Gaswechsel* in Nagel's *Handbuch der Physiologie des Menschen.*
2. *Kolloid Zeitschr.*, III, 169, 1908.
3. *Ibid.*, II, 264, 1908.
4. *Loc. cit.*
5. *Trans. Chem. Soc.*, XCVII, 536, 1910.

in the few cases in which an increased solubility was observed, it seemed most suitable to attribute the effect, except in the case of charcoal and silicic acid, to chemical combination.

In order to compare the behaviour of blood and blood-serum with the behaviour found in the case of the colloids previously investigated, we carried out a few determinations of the solubility of oxygen, carbon dioxide, carbon monoxide, nitrous oxide, and nitrogen in defibrinated ox blood and ox serum, under pressures varying from that of the atmosphere up to about 1,400 mm. of mercury.

The method employed by most physiologists for determining the amount of gas absorbed by blood or serum has been to pump off the dissolved gas and to measure the volume of gas so pumped off. We have adopted the opposite course, and have measured the volume of gas *taken up* by the liquid under different pressures.[1] Since the solubility of a gas is expressed by the ratio concentration of gas in liquid to concentration of gas in the gas space, it follows that since these two factors increase together with increase of pressure for gases obeying Henry's law, the solubility-pressure curve will in such cases be a straight line parallel to the pressure axis. It differs, therefore, from the curve of absorption, which is a continuously ascending curve. This point requires to be emphasised, since we have employed the solubility co-efficients, whereas biologists use, as a rule, the absorption co-efficients.

The temperature at which the solubility was determined was, in all cases, 25·0°, and as absorbing liquids we used 'natural' ox blood and ox serum as obtained from the slaughter-house, as well as 'deaërated' blood and serum, i.e., blood or serum which had been kept under a reduced pressure of about 15 mm. of mercury for an hour.

I. Solubility of Carbon Dioxide

For the purpose of these experiments commercial carbon dioxide was employed, containing 0·58 per cent. of impurities. The solubility in pure water was found to be 0·817, the solubility being, in accordance with Henry's law, independent of the pressure. The solubility in blood and serum is given in Table I, the results being also exhibited graphically in fig. 1.

1. For a description of the apparatus employed and the formula for calculating the solubility, see *Geffchen, Zeitschr. physikal. Chem.*, XLIX, 298, 1904; Findlay and Creighton, *Trans. Chem. Soc.*, XCVII, 537, 1910.

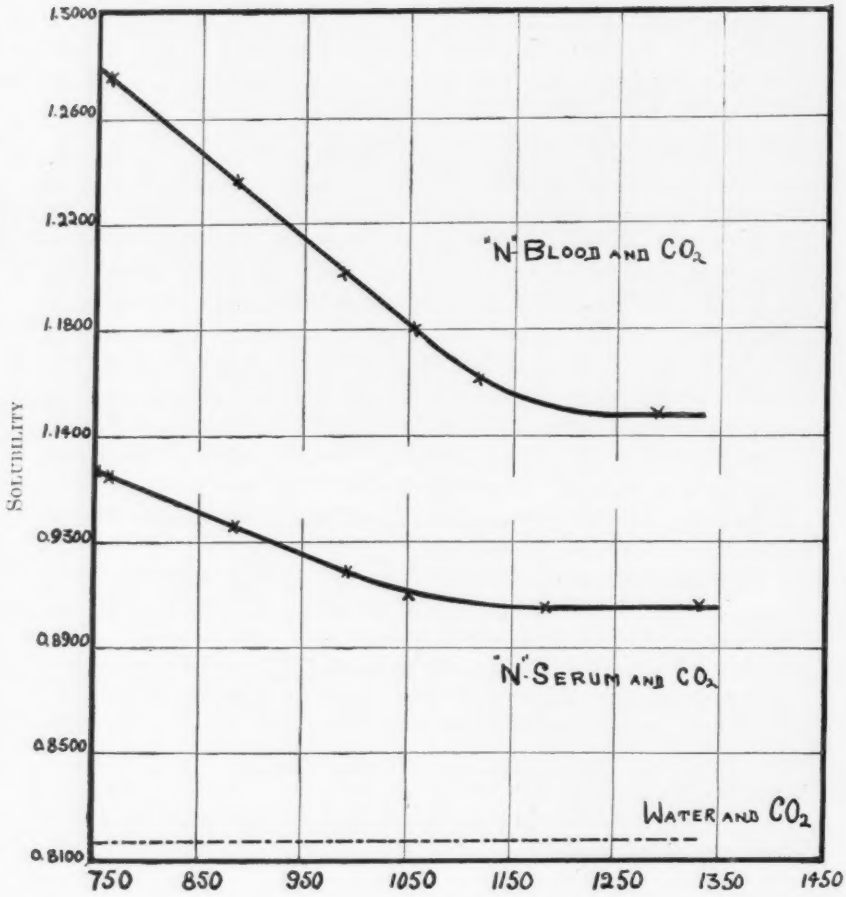

FIG. 1.—PRESSURE IN MM. OF MERCURY

TABLE I

		'Natural' Ox Blood. Density 1·067				
Pressure in mm. ...	762	882	985	1,050	1,115	1,291
Solubility of CO_2 ...	1·276	1·236	1·201	1·181	1·162	1·149
		Deaërated Ox blood. Density 1·067				
Pressure	758	850	937	1,061	1,232	1,301
Solubility	1·461	1·401	1·362	1·309	1·245	1·226
		Natural Ox serum. Density 1·025				
Pressure	761	879	989	1,050	1,179	1,331
Solubility	0·956	0·935	0·919	0·910	0·906	0·907

In all these cases both the blood and the serum quickly became saturated with the gas, and even after ten or twelve hours no further quantity of gas was taken up.

II. SOLUBILITY OF OXYGEN

Gas obtained by heating pure potassium permanganate; passed over soda lime. The solubility in pure water (mean of five series) was found to be 0·0294. Geffchen[1] found 0·0308; Bohr and Bock,[2] 0·0317; Winkler,[3] 0·03097.

TABLE II. Solubility of Oxygen in Blood and Serum

		Natural ox blood. Density 1·064				
Pressure in mm.	756	918	1,061	1,243	1,342	1,481
Solubility	0·1180	0·1054	0·0957	0·0861	0·0832	0·0779
		Natural ox serum. Density 1·027				
Pressure in mm.	752	871	1,003	1,192	1,346	1,523
Solubility	0·0016	0·0028	0·0042	0·0067	0·0081	0·0091
		Deaërated ox serum. Density 1·027				
Pressure in mm.	746	901	1,187	1,256	1,390	1,551
Solubility	0·0056	0·0081	0·0123	0·0135	0·0162	0·0183

See also Figs. 2 and 3. In the figures E-blood and E-serum represent deaërated blood and serum.

The point of saturation was quickly reached, and no further absorption occurred after a period of twelve hours.

1. *Zeitschr. physikal. Chem.*, XLIX, 298, 1904.
2. *Wied. Annalen*, XLIV, 318, 1891.
3. *Ber.*, XXII, 1764, 1889.

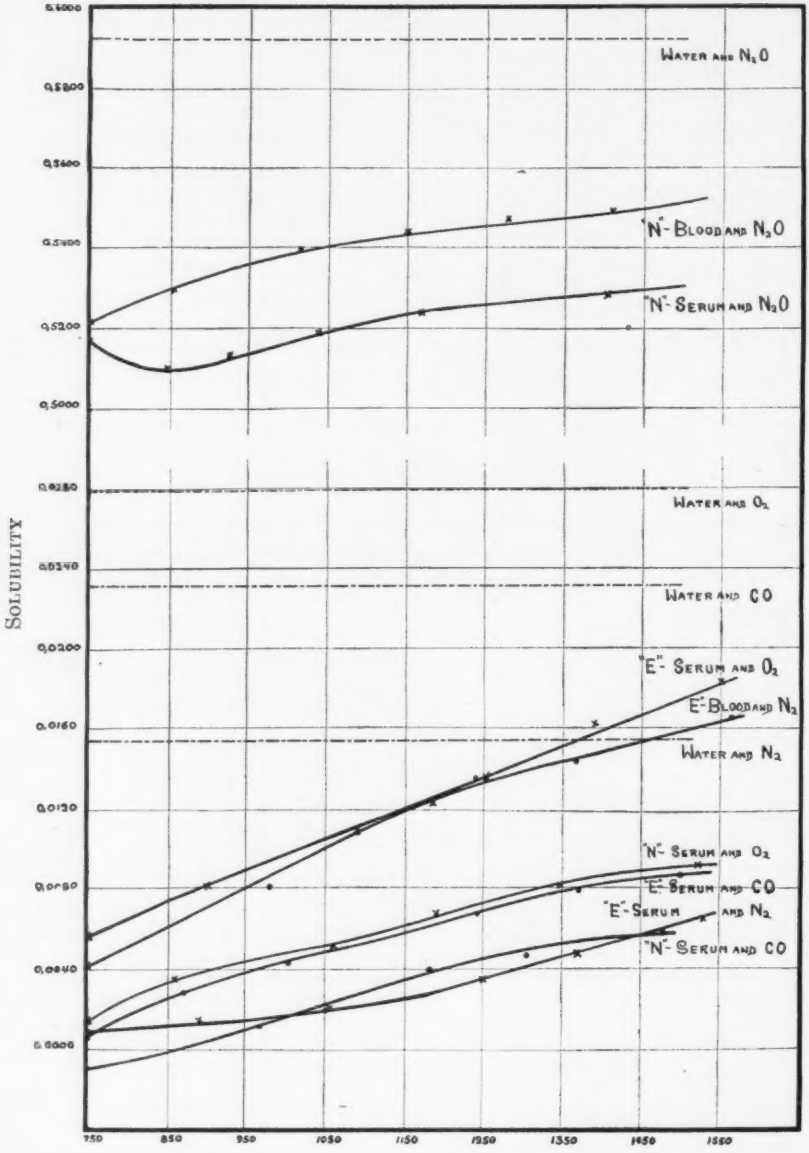

Fig. 2.—Pressure in MM. of Mercury.

III. Solubility of Carbon Monoxide

The gas was prepared by heating pure potassium ferrocyanide with concentrated sulphuric acid, and precautions taken to free it from air. The solubility in pure water was found to be 0·0231. Winkler[1] found 0·02338.

Table III. Solubility of Carbon Monoxide in blood and serum

			Natural ox blood.	Density 1·061			
Pressure in mm.	751	944	1,144	1,371	1,434	1,528
Solubility	0·0979	0·0828	0·0745	0·0658	0·0657	0·0641
			Deaërated ox blood.	Density 1·061			
Pressure in mm.	751	871	1,056	1,140	1,274	1,543
Solubility	0·0949	0·0826	0·0746	0·0710	0·0661	0·0582
			Deaërated ox serum.	Density 1·028			
Pressure in mm.	754	859	1,061	1,243	1,372	1,509
Solubility	0·0014	0·0035	0·0051	0·0066	0·0078	0·0086

See also Figs. 2 and 3.

IV. Solubility of Nitrous Oxide

The gas was prepared by heating pure ammonium nitrate, and was freed from air and purified by passing through solutions of potassium hydroxide and ferrous sulphate. The solubility in pure water was found to be 0·592. Geffchen[2] found 0·594.

Table IV. Solubility of nitrous oxide in blood and serum

		Natural ox blood.	Density 1·065			
Pressure in mm.	745	854	1,012	1,152	1,277	1,408
Solubility	0·521	0·530	0·539	0·544	0·547	0·548
		Natural ox serum.	Density 1·025			
Pressure in mm.	737	846	925	1,036	1,169	1,402
Solubility	0·517	0·509	0·513	0·519	0·524	0·528

See also Fig. 2.

1. *Ber.*, XXXIV, 1408, 1901.
2. *Loc. cit.*

V. Solubility of Nitrogen

The gas was prepared by heating a mixture of sodium nitrite and ammonium chloride, and was carefully purified. Its solubility in pure water was found to be 0·0154. Bohr and Bock[1] found 0·0164.

Table V. Solubility of nitrogen in blood and serum

			Deaërated ox blood.		Density 1·066		
Pressure in mm.	757	979	1,089	1,241	1,367	1,564
Solubility	0·0041	0·0080	0·0108	0·0135	0·0143	0·0165
		Deaërated ox serum.		Density 1·028		
Pressure	734	891	1,056	1,250	1,368	1,529
Solubility	0·0008	0·0014	0·0020	0·0034	0·0047	0·0065

See Fig. also 2.

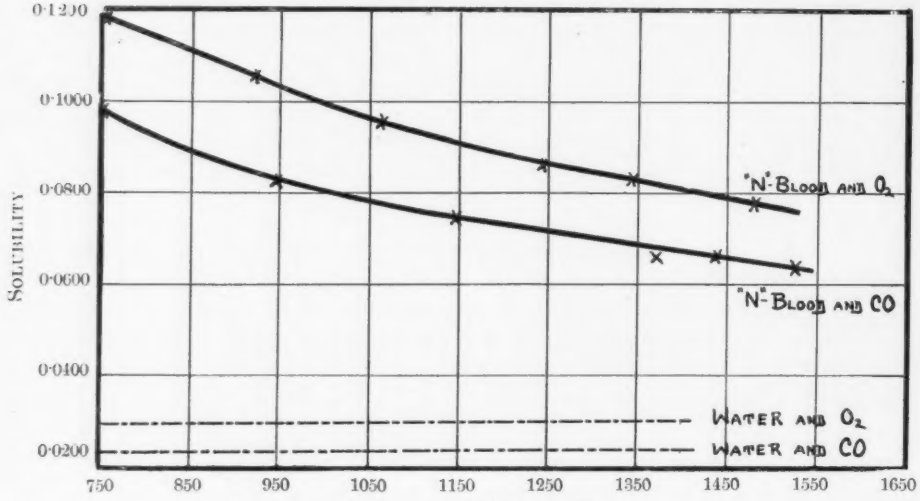

Fig. 3.—Pressure in MM. of Mercury.

Discussion of Results

It will be seen from an examination of the data obtained that the solubility phenomena observed are of two kinds. On the one hand it will be seen that the solubility of carbon dioxide, oxygen and carbon monoxide in blood is greater than in water; but that the solubility diminishes at a decreasing rate as the gas pressure is increased. A similar behaviour is

1. *Loc. cit.*

found with respect to the solubility of carbon dioxide in serum. On the other hand, it will be noticed that the solubility of nitrous oxide and nitrogen, both in blood and in serum, is less than in the case of pure water. Moreover, it will be observed that in these cases the solubility curve rises with increase of pressure, so that at high pressures it may even rise above the curve for pure water, as in the case of nitrogen and blood.

With the single exception of carbon dioxide in serum, the solubility of all the other gases is less in serum than in water, and the solubility-pressure curves are the same in type, and roughly parallel to one another. The behaviour is quite analogous to that found previously in the case of the solubility of carbon dioxide and nitrous oxide in presence of various colloids between which and the gases chemical combination seemed excluded. Quite analogous also is the rise in the solubility-pressure curve.

Since in these cases the diminished solubility of the gases in serum as compared with that in pure water appears to exclude the idea of chemical combination, and since the similarity of the solubility curves seems to point to the absence of any marked specific action, one is led to the conclusion that the continued rise in the solubility-pressure curve is to be ascribed to some physical interaction, such as surface condensation or adsorption. We are not concerned at the present moment with the real nature of these physical actions, and merely wish to indicate that the influence producing the rise in the solubility-pressure curve is most probably not a chemical combination in the usually accepted sense.[1]

The rise in the solubility-pressure curve must, we believe, be ascribed to the influence of the colloidal material in the serum. Although the solubility of carbon dioxide has been determined in solutions of different salts and non-electrolytes,[2] in no case has an ascending solubility curve been obtained except in the case of colloidally dissolved substances.

We find also that these different solubility-pressure curves follow the general adsorption formula $C_2^4/C_1 = K$, where C_2 represents the gas concentration in the liquid, and C_1 the concentration in the colloid phase. This is shown by the following table, which refers to the curve for carbon

1. Moore and Bigland, *Bio-Chemical Journal*, V, 64, 1910, suggest the term *colloidal reaction* to a chemical process where a colloid takes part in a reaction, and reserve *adsorption* for the purely physical process. In the absence of specific chemical action, the behaviour observed here would be regarded as due to *adsorption*.

2. A number of these determinations have been made in this laboratory by Mr. B. Shen, but the results have not yet been published.

monoxide and deaërated serum. In making the calculation it has been assumed that the gas absorbed at 750 mm. pressure represents C_1; that is, it is assumed that no adsorption occurs at this pressure. This assumption is no doubt wrong, but the error introduced will affect only the absolute value, not the constancy of K.

Pressure	Solubility	C_1	C_2	$\frac{C_2^4}{C_1} \times 10^{10}$
750	0·0014	—	—	—
850	0·0032	0·000251	0·000196	0·058
950	0·0042	0·000437	0·000219	0·052
1,050	0·0051	0·000639	0·000242	0·053
1,150	0·0060	0·000810	0·000265	0·057
1,250	0·0070	0·001151	0·000288	0·060
1,350	0·0078	0·001421	0·000311	0·066

Similar series of values are obtained in the case of the other curves, with the single exception of that for nitrogen in deaërated serum, in which case there is a gradual fall in the value of C_2^4/C_1. The fact that the nitrogen curve is of the same type as the others indicates, however, that the gradual fall in the value of C_2^4/C_1 is probably due to experimental errors or to the fact that a slightly different value should be chosen for the index of C_2.

Whereas, therefore, we believe that the solubility relations in the case of oxygen, carbon monoxide, nitrogen and nitrous oxide in serum, and of the last two gases also in blood, are explicable on the basis of adsorption, no such explanation is sufficient to account for the absorption of oxygen, carbon monoxide and carbon dioxide in blood, and of the last-mentioned gas also in serum.

In these cases the solubility of the gases is markedly greater than in water, and the solubility curves *fall* with increase of pressure. To explain such curves as these requires something more than an assumption of surface adsorption.

With regard to the absorption of oxygen by blood two views are held. On the one hand there is the view, held by the majority of physiologists,[1] that the absorption of oxygen by blood is due mainly to the formation of one or more dissociable compounds; on the other hand, there is the view, represented chiefly by Wo. Ostwald,[2] that the chief factor concerned is adsorption, or a surface action. As already pointed out, this view was

1. See articles by Bohr, already referred to; also Moore and Bigland, *Bio-Chemical Journal*, V, 65, 1910. Barcroft and Camis, *Jour. Physiology*, XXXIX, 130, 1909.

2. *Loc. cit.*

also suggested by Findlay and Harby to explain the solubility of carbon dioxide in blood, but the investigation by the present authors of the influence of colloids on the absorption of gases, has led us to abandon this view, and to accept the 'chemical' explanation. Adsorption, we believe, does exercise an influence, but this influence appears to be of secondary importance.

It is already well known that as the oxygen-pressure increases, the absorption gradually decreases, and that between the pressures of 150 and 760 mm. only a very small extra amount of oxygen is taken up by blood (Bohr). From this behaviour Bohr concluded that the maximum degree of combination of oxygen and haemoglobin had occurred at pressures of about 150 mm.

From the curve shown in fig 3, it will be seen that although the solubility of oxygen in blood is greater than in water it decreases as the pressure is raised.[1] The behaviour is, however, quite explicable, provided we assume, and the assumption seems justified by the work of Bohr and others, that the 'chemical combination' is practically complete at the initial pressure of 760 mm. In this case the greatest volume of gas (measured under the particular pressure employed) would be absorbed initially. As the pressure is increased further absorption would be (practically), in accordance with Henry's law, influenced to a certain extent by adsorption; but as the *amount* of combined gas remains practically constant, there would be a decrease in the total volume of gas absorbed, the volume always being measured under the pressure at which the solubility is determined.

That this explanation is at least a plausible one is shown by the following calculation. If the increase in the solubility of oxygen in blood as compared with water, be due principally to chemical combination, then the difference between the solubility in water and in blood at atmospheric pressure should give approximately the amount of oxygen that is chemically combined. Then, knowing the volume of gas absorbed by water, and that absorbed by an equal volume of blood at atmospheric pressure, the volume of oxygen absorbed by blood at any other pressure should be given by the formula—

$$V = \frac{v_1 \times p_0}{p_1} + v_2 + v_3$$

Here v_1 is the volume of gas chemically combined in 1 c.c. of blood at the initial pressure p_0; p_1 is the pressure at which the solubility is to

1. The *solubility* decreases, but of course the actual *amount* absorbed increases.

be determined; v_2 is the volume of gas dissolved by 1 c.c. of water at the initial pressure, and v_3 is a volume correction for gas adsorbed.

Thus, in the case of oxygen and natural ox blood, the volume of oxygen combined in 1 c.c. of blood is equal to 0·1180 – 0·0294 = 0·0886 c.c. Then the calculated solubilities at different pressures will be as follows: —

Pressure	$\frac{v_1 p_0}{p_1}$	v_2	v_3	V	
				Calculated	Found
756	0·0886	0·0294	—	—	—
918	0·0730	0·0294	0·002	0·1044	0·1054
1,061	0·0633	0·0294	0·003	0·0957	0·0957
1,243	0·0538	0·0294	0·005	0·0882	0·0861
1,342	0·0498	0·0294	0·0065	0·0857	0·0832
1,481	0·0453	0·0294	0·007	0·0817	0·0779

The value of v_3 was taken from the solubility curve of oxygen in 'natural' serum.

The agreement shown in the above table between the solubility values calculated and found, may be regarded as very satisfactory.

The chemical explanation of the absorption of oxygen by blood is supported also by the fact that the solubility curve for oxygen and blood is of precisely similar form to that for carbon monoxide and blood. That in this case there is chemical combination between the carbon monoxide and the haemoglobin has probably never been called in question.

Just as we conclude that the increased solubility of oxygen in blood is mainly due to chemical combination, so also must we attribute the increase in the solubility of carbon dioxide in blood and also in serum, to the same cause. This view is in harmony with the experimental work of several physiologists.[1] It is true that several investigators[2] have, by electrical measurements and by means of indicators, obtained results which point to the blood serum being 'water-neutral,' but in view of other experimental work it seems certain that blood and serum do have power to combine with carbon dioxide,[3] and the results obtained by us certainly strengthen the chemical explanation of carbon dioxide absorption.

It may perhaps be recalled that the solubility measurements with oxygen were carried out only with 'natural' ox blood, as indeed has

1. See Boycott and Chisholm, *Bio-Chemical Journal*, V, 23, 1910.

2. Hoeber, *Pflügers Archiv.*, XCIX, 572, 1903; Farkas, *ibid.*, XCVIII, 551, 1903; Friedenthal, *Verworns Archiv. f. allgem. Physiologie*, IV, 44, 1904; van Westenryk, *Arch. exp. Path. Pharm. Suppl.*, p. 517, 1908.

3. Reference may be made to the work of Siegfried, *Zeitschr. physiolog. Chem.*, XLIV, 85, 1905; XLVI, 491.

been customary with other investigators. Although we regret that we did not also determine the solubility in deaërated blood, we do not think that the use of 'natural' blood invalidates in any way the conclusions we have arrived at. Our main purpose was to determine the *form* of the solubility-pressure curve, which our previous investigation had shown us to be of importance; but although higher values would have been obtained for the solubility of oxygen in deaërated blood, the experiments with carbon dioxide and carbon monoxide show that the *form* of the curve would still have remained the same.

It ought perhaps also to be pointed out that the conditions under which we worked (comparatively high pressures), were very different from those under which absorption of oxygen or of carbon dioxide takes place naturally. Whether, at lower pressures, the relative importance of chemical combination and of adsorption might alter, it is not possible at present to say. So far the solubility of gases in presence of colloids at pressures below atmospheric pressure, has not been determined. We hope, however, to extend the investigation in this direction.

Summary

1. The solubility of oxygen, carbon dioxide, carbon monoxide, nitrous oxide, and nitrogen in ox blood and ox serum has been determined at a temperature of 25° and under pressures varying from 760 to 1,400 mm.

3. The solubility of oxygen, carbon monoxide, and carbon dioxide in blood, and of carbon dioxide in serum, is greater than in water; the solubility of nitrogen and nitrous oxide in blood and in serum, and of oxygen and carbon monoxide in serum is less than in water.

3. Where the solubility is increased, the solubility curves fall with increasing pressure; where the solubility is diminished, the solubility curve rises with increase of pressure.

4. The increased absorption of oxygen, carbon monoxide and carbon dioxide in blood, and of carbon dioxide in serum, is to be ascribed in the main to chemical combination.

5. In the cases of diminished solubility, the rise in the solubility curve is to be ascribed to adsorption.

TRIMETHYLAMINE AS A NORMAL CONSTITUENT OF HUMAN BLOOD, URINE AND CEREBROSPINAL FLUID

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In the course of some experiments undertaken with a view to ascertain the variation of trimethylamine in the urine in cases of nervous disease, we have modified the methods for the estimation of trimethylamine in urine in such a way as greatly to accelerate the process. The following paper contains an account of this method, together with observations on blood and cerebrospinal fluid, which go to prove that trimethylamine is normally present in these fluids.

The method of de Filippi[1] for the estimation of trimethylamine in urine, which has subsequently been employed by Bauer,[2] Caccio,[3] and one of us,[4] depends upon the collection of the total volatile alkali from the urine by distillation with strong caustic potash, the evaporation of the mixed ammonium chlorides in the distillate, and the repeated extraction of these with alcohol, which removes the hydrochlorides of the methylamines and leaves the bulk of the ammonium chloride behind. The final alcoholic extract is then evaporated to dryness, the residue dissolved in water, and treated in a special all-glass distillation apparatus with sodium hypobromite. By careful preliminary experiments, which were confirmed by Bauer, de Filippi proved that all the bases present, except trimethylamine, are decomposed by this reagent. Before distillation, however, it is necessary to remove the excess of hypobromite, and this is done by acidifying and distilling off the bromine liberated. The liquid remaining in the flask is then again made alkaline, and the trimethylamine distilled off and collected in standard acid. These processes are extremely tedious, some two and a half days being required for each estimation, and this delay militates against the use of the

1. Filippo de Filippi, *Zeitschr. physiol. Ch.*, 433, 1900.
2. Bauer, *Beitrage*, 11, 502.
3. Caccio, *International Congress of Applied Chemistry, London*, 1909.
4. Golla, *Quarterly Journal of Medicine*, II, 231, 1909.

method for experiments on the metabolic variations of the trimethylamine output in the urine under varying conditions of diet.

We therefore modified the method in several important directions, which render the process much more expeditious and at least as accurate as the original one. Our modifications consist in :—(*a*) The distillation of the urine in a vacuum at a low temperature, so that only the free ammonia and the trimethylamine are obtained in the distillate. The troublesome process of repeated extraction of the evaporated distillate with alcohol to separate the methylamines from the enormous quantity of ammonium chloride obtained by the original method, is now replaced by a single extraction, as comparatively little ammonium chloride has to be removed. (*b*) After treatment of the mixed ammonium and methylamine hydrochlorides with sodium hypobromite, the excess of hypobromite is destroyed by the addition of sodium arsenite solution. This reagent reacts instantly, and avoids the troublesome distillation of free bromine in a special all-glass apparatus as in de Filippi's process.

The procedure followed by us may be detailed as follows :—

Taking advantage of the fact shown by Schlösing that urine, heated *in vacuo* with baryta water at a temperature below 40° C. gives off only its free ammonia, and having satisfied ourselves that small quantities of trimethylamine could be determined in a similar way, we place one and a half to three litres of urine in a strong five-litre bolthead flask, and add milk of baryta till the reaction is permanently alkaline. A few pieces of pumice, to ensure even boiling, are thrown in, and about 5 c.c. of purified paraffin oil added to prevent the liquid frothing. We found the so-called odourless paraffin very effective for the purpose. Some of the paraffin distils over, but its presence in the distillate causes no trouble. The bolthead flask is connected with a long condenser, the end of which dips into a large receiver flask. The latter is connected with two smaller wash-bottles containing hydrochloric acid, then to a trap bottle, and then to a powerful water pump. The absorption apparatus is placed in a box and surrounded with a good freezing mixture. The urine is distilled at a temperature of from 25° to 30° C. and under a pressure of about 20 mm. for two to three hours. From one and a half litres of urine we obtain about 500 c.c. of distillate, which is washed out and evaporated to dryness. To economise hypobromite it is advantageous to extract the residue with alcohol, filter, evaporate the extract to dryness, and again extract with a little alcohol. The aqueous solution is then placed in a small distillation flask, and connected with a condenser dipping into a receiver

which is fitted with a side U-tube containing a little strong hydrochloric acid. The bulk of the distillate solution need not be large. Sodium hypobromite is added through a dropping funnel till a permanent golden colour remains. Air is now drawn through the whole apparatus, being admitted through the tap of the dropping funnel, and the traces of trimethylamine liberated by the alkali of the hypobromite solution are thus collected in the acid of the U-tube. The excess of hypobromite is now removed by the addition of an alkaline solution of sodium arsenite, and the distillation is completed in the usual way, the amount of trimethylamine being estimated by titration of the neutralised acid, methyl orange being used as an indicator. To ensure good results the following points are worthy of note:—(1) During the vacuum distillation the receiver should be thoroughly cooled. (2) The distillate should be reduced to small bulk before treatment with the hypobromite solution. (3) The hypobromite should be fresh. It is most important that in its preparation the temperature should be kept down to zero during the addition of the bromine to the sodium hydrate solution. (4) If any doubt exists as to the presence of ammonia in the final distillate a few drops of this may be tested with Nessler's reagent, which gives no colour or precipitate with a dilute solution of trimethylamine.[1]

An estimation of the trimethylamine in 1,500 to 2,000 c.c. of urine can be carried out by this process in about four hours, but good results can be obtained with a litre of urine in considerably less time. The free ammonia of the urine can be determined simultaneously by using a known excess of acid in the first distillation process. Thus 1,500 c.c. of fresh normal urine gave total volatile alkali equivalent to 365 c.c. decinormal sodium hydrate solution. Of this the trimethylamine was found to be equivalent to 3·7 c.c., so that the free ammonia was equivalent to 361·3 c.c. These figures give for 1,500 c.c. of urine a free ammonia content of 0·6142 gram (0·0409 per cent.), and trimethylamine 0·02183 gram—numbers which are in close agreement with those found by other observers and by ourselves, for normal urine.

In order to compare the results obtained by the modified method with that of the original de Filippi process, we made the following parallel

1 See Delépine, C.R. CXXII, 1064, 1172, 1896, who has shewn that pure di- and trimethylamines give white precipitates with Nessler's reagent which disappear on the addition of water. This we can confirm. Bauer (*loc. cit.*), however, speaks of a brown coloration with trimethylamine. Monomethylamine gives a yellow precipitate which does not disappear on dilution.

experiments. A large sample of fresh normal urine was divided into equal parts for each comparison.

(1) One and a half litres of urine gave by the above method 0·020 gram of trimethylamine; by that of de Filippi 0·018 gram.

(2) Two litres of urine gave by the above method 0·025 gram of trimethylamine; by that of de Filippi 0·020 gram.

(3) One and a half litres of urine gave by the above method 0·017 gram of trimethylamine; by that of de Filippi 0·015 gram.

The results are sufficiently close, and, if anything, point to the fact that less loss occurs in our process than in the more lengthy one of de Filippi.

Reactions of and Tests for Trimethylamine

The discovery of Mott and Halliburton[1] that choline occurred in the cerebrospinal fluid of patients suffering from degenerative nervous diseases led to the investigation of various tests for the detection of minute quantities of that substance. Rosenheim,[2] who worked the matter out carefully, recommends three reagents for this purpose, which have since been extensively employed. These are (*a*) Potassium periodide (Florence's test); (*b*) alloxan—a colour test; (*c*) Bismuth periodide (Dragendorff's reagent as modified by Kraut[3]). The macro- and microchemical reactions of pure choline and pure neurine have been tabulated by Gulewitsch,[4] who has also stated the limits of sensitiveness of the above reagents, and also those of phosphotungstic and phosphomolybdic acids, picric acid, etc.

In order to be in a position to detect and differentiate minute quantities of trimethylamine such as might conceivably be obtained from cerebrospinal fluids, we made an examination of the action of the above-mentioned reagents on trimethylamine. The pure hydrochloride obtained from Kahlbaum was employed. Its solution gave no coloration with Nessler's reagent. For most experiments it was, moreover, purified by treatment with sodium hypobromite and distillation into pure hydrochloric acid. As a result of the experiments made by us it was found that trimethylamine in very small quantities behaves with the reagents

1. Mott and Halliburton, *Phil. Trans.*, 4, 1899
2. Rosenheim, *J. Physiol.*, XXXIII, 221.
3. Kraut, *Ann. d. Chem.*, CCX, 310.
4. *Zeit. physiol. Chem.*, XXIV, 514; XXVI, 175.

employed almost exactly as does choline, and that whereas slight differences manifest themselves when comparatively strong solutions are employed, with very dilute solutions there is practically no difference in the reactions of the two substances. It is thus rendered possible that some of the 'choline' previously found in various fluids and tissues may have consisted of trimethylamine.

Our observations are as follows:—

1. *The Alloxan Test.* Parallel tests with purified hydrochlorides of choline and trimethylamine were made, using a strong solution of alloxan. The rose-red colour turning to deep blue-violet on addition of potassium hydrate was given by both substances, and it was not found possible to differentiate them in this way.

2. *The Bismuth Iodide Test.* The solution used was prepared according to the directions of Kraut, as this was found by Rosenheim to be more sensitive towards choline than the original Dragendorff's reagent. We made it, however, half as strong again as required in Kraut's formula, and for microscopic tests with minute amounts of substances we found it best to add the reagent directly to the solid residue to be tested. The presence of water seems to disturb the equilibrium of the reagent, and is apt to modify the formation of the crystals. 0·1 to 0·05 c.c. of solutions of choline and trimethylamine of known strengths were evaporated to dryness at a very gentle heat on a microscopic slide, and the dried residue covered with a slip and treated with a drop of the reagent. 0·02 milligram of trimethylamine hydrochloride thus deposited on a slide gave a distinct smell of trimethylamine on treatment with one drop of potassium hydrate solution, and the same weight treated with Kraut's solution gave a well-marked yellowish-red colour visible to the naked eye, whilst under the microscope short six-sided prisms and column-shaped crystals were observed which were indistinguishable from those given by an equal amount of choline. The macroscopic appearance of the choline precipitate, however, is a bright red as distinguished from the yellowish-red of trimethylamine. Crystallisation of the choline precipitate also takes place less readily than in the case of trimethylamine and the frequency with which an amorphous precipitate is obtained with choline led us at first to hope that the test would constitute a distinction between the two substances. This hope, however, proved to be vain, as when crystallisation occurs the two precipitates are indistinguishable.

3. *The Potassium Periodide Test (Florence's Test).* The proportions required for this reagent are given by Florence as Potassium iodide : iodine : water, as 5 : 8 : 100 (i.e. approximately the proportions required by the formula KI_3); whilst Rosenheim, who used it extensively as a test for choline, gives the proportions 6 : 2 : 100. We found the proportion 4 : 4 : 100 most convenient. This test is extremely sensitive, both for choline and trimethylamine. We found after careful comparison using the dry slide method, that crystals formed by choline periodide do not vary in appearance, whether there was much or little choline present. Choline always showed the characteristic isolated rectangular needles, shaped like haemin crystals, which are figured by Rosenheim.[1] On adding a drop of the reagent to the dry choline salt innumerable droplets are observed, which rapidly crystallise. The crystals are, however, more stable than those which appear to have been obtained by Rosenheim, and when once they have resolved into drops we did not succeed in reforming them. When a solution of trimethylamine hydrochloride containing as much as 0·02 milligram of the salt is evaporated on a slide and treated with a drop of the reagent, an immediate formation of black oily drops takes place which rapidly turns to crystals visible to the naked eye. Under a low power (fig. 1) these are seen to consist of dagger-shaped crystals, which group themselves in a very characteristic cross shape. When smaller amounts of trimethylamine, such as about 0·001 milligram are used, a cloud of black drops is immediately observed on the addition of the reagent, and these more or less quickly crystallise, forming minute rod-like crystals (fig. 2), which very closely resemble those of choline (fig. 4). It will be seen from the photographs that in the highly dilute solutions used it is practically impossible to differentiate between choline and trimethylamine by this reaction.

Composition and Properties of Trimethylamine Periodide

The remarkable ease with which the crystals of trimethylamine periodide are formed, even in very dilute solution, led us to examine them more closely with a view to ascertaining whether their formation could not be employed as a method for the estimation of small quantities of trimethylamine. Although a great deal of information has been collected with regard to the solid organic polyiodides,[2] the data refer to

1. *J. Physiol.*, XXXIII, 222.
2. Abegg, *Zeit. anorgan. Chem.*, L, 403 (1906). Geuther. *Annalen*, CCXL, 66.

iodides of the type $NR_4I.I$, and the characteristic crystalline derivative found in Florence's test does not seem to have been examined or its crystalline nature recognised by physiological workers.[1] Staněk[2] states that choline periodide has the formula $C_5H_{14}NOI.I_8$, agreeing in this respect with the opinion of Abegg,[3] that all these organic iodides yield a periodide of the formula $R.I_9$. This choline enneaiodide described by Staněk is formed quantitatively, and is permanent, so that it can be washed, dried, and the nitrogen content determined by Kjeldahl's process. The periodide of trimethylamine did not prove so satisfactory. Trimethylamine periodide was apparently first described by J. Weiss, and in a communication by Schmidt[4] some analytical data are given which point to a formula $NMe_3HI.I_4$. The substance was also prepared by Delépine,[5] who stated that he obtained figures which supported this conclusion. We have not, however, been able to find any very precise account of this body, and of the conditions under which it was formed. It was important, for example, to ascertain whether the composition of the crystals varies with the iodine concentration of the reagent; whether, seeing that they are generally prepared from the hydrochloride, they were free from chlorine, and also under what conditions, if any, they were stable.

We have investigated these points, and have arrived at the following conclusions:—On the addition of a strong solution of trimethylamine hydrochloride to an excess of the reagent a brown precipitate forms which rapidly resolves itself into a deposit of large flake-like crystals showing a blue-green dichroic effect. These can be filtered off through glass wool on the pump, and washed with a little ice-cold water to remove excess of the reagent. In the moist state, however, water continues to abstract iodine from them, and it is not possible to obtain a wash water free from iodine, though the loss, when very cold water is used, is very small. The crystals were allowed to dry on a tile and found to be comparatively stable in the air, a slight loss of iodine taking place in warm weather. All the usual organic solvents extract iodine from them though very slowly. Benzene appears to act most rapidly as a solvent, whereas pentane of b.p. 60° C. is almost without solvent action. The crystals,

1. *Cf.* Staněk, *Zeit. physiol. Chem.*, XLVII, 83, 1906.
2. Staněk, *ibid.*, XLVI, 280, 1905; XLVII, 83; XLVIII, 334, 1906.
3. *Loc. cit.*, 405.
4. Schmidt, *Annalen*, CCLXVII, 258, 1892.
5. Delépine, *Annales de Chim. et de Phys.*, vii. VIII, 439, 1896.

if pressed with a glass rod partially liquefy, forming a semi-solid mass, but harden and crystallize again on being left. When slowly heated they fall together to a black pasty fluid at about 66° C., and decompose between 150° and 180° C. On ignition no residue is left, so that the compound contains no potassium, and, as will be seen below, it is free from chlorine. An analysis by Carius' method of a sample washed with a little ice water, dried and washed with pentane, gave the following result:—

0·4837 gave 0·8059 AgI—Iodine found 90·2 per cent.
$NMe_3HI.I_3$ requires 89·4 per cent, $NMe_3HI.I_4$ requires 91·34 per cent, and $NMe_3HI.I_5$ requires 92·72 per cent of iodine.

To obviate the uncertainty of drying the crystals for analysis the ratio NMe_3 : I in the freshly-formed product was determined in the following way. The crystals were washed with small quantities of ice water till the filtrate was very pale. They were then put into a large flask connected with a condenser and receiving apparatus charged with standard acid for collecting the trimethylamine. Pure potash solution was then run into the flask, and the solution gently warmed till the crystals had dissolved to a clear solution. The trimethylamine was distilled off and estimated as usual.

The liquid remaining in the flask contained iodide and iodate of potassium. It was made up to a known volume, acidified, and a portion titrated with sodium thiosulphate, no difference in the value being obtained if potassium iodide was previously added.

Another measure of the liquid after acidification was extracted with chloroform to remove the iodine, and as it was found that the solution still contained potassium iodide, the iodine in this was estimated by treatment with hydrogen peroxide, followed by extraction with ether. The sum of these two values gave the total iodine. The liquid left after the removal of the iodine by ether gave no precipitate with silver nitrate, so that chlorine was absent. Some of the results obtained are as follow:—

Specimen 1. Made from reagent 4 KI : 4 : 100. Crystals washed four times with ice water.
Specimen 2. Reagent 6 KI : 2 : 100.
Specimen 3. Reagent 4 KI : 1·6 : 100. (Proportions given in grams, not gram molecules).

Found	NMe_3 in grams	Iodine in grams	Atoms of Iodine combined with NMe_3
1. ...	0·18821 ...	2·1209 ...	5·22
2. ...	0·13570 ...	1·4927 ...	5·26
3. ...	0·08251 ...	0·90598 ...	5·04

It thus appears that there are five atoms of iodine present combined with each trimethylamine molecule. To ascertain whether the body was of the type NMe_3I_5, or whether it was of the type $NMe_3HI.I_4$, the following experiments were made:—

Preparation A. A strong reagent (4 : 4 : 100) was made and the crystals formed were washed with a little ice water and dried over sulphuric acid. They dissolved very slowly in 20 per cent. potassium iodide solution, but on the addition of benzene formed a greasy mass which slowly went into solution.

0·3164 required 17·89 c.c. N/10 thiosulphate. Iodine found 71·8 per cent.

Preparation B. Same method and treatment.

0·7781 required 43·78 c.c. N/10 thiosulphate. Iodine found 71·5 per cent.

Preparation C. made from weak reagent (4 KI : 1·6 : 100). 0·4308 required 27·36 N/10 thiosulphate. Iodine found 72·3 per cent.

$NMe_3HI.I_5$ requires 67·08, $NMe_3HI.I_4$ 73·09 and $NMe_3HI.I_3$ 77·4 per cent. of iodine.

These results are in agreement with the constitution $NMe_3HI.I_4$. We prepared the same body from trimethylamine hydriodide, so that when the hydrochloride is used the potassium iodide must convert the hydrochloride to the hydriodide in the first instance. It would then appear that variations in the method of preparation do not affect the composition of the crystals.

As the crystals seemed to have a definite composition under varying conditions of preparation, we next carried out a number of experiments to ascertain whether their formation could not be utilised as a method for the estimation of trimethylamine. As it was not possible to collect and weigh them, and as the Staněk process used for choline required larger quantities of the base than were likely to be contained in a litre of urine, we endeavoured to measure the absorption of iodine from the reagent on the addition of known quantities of trimethylamine.

For this purpose a solution of trimethylamine hydrochloride containing 7·3 grams per litre was used. Quantities of 25 c.c. of the periodide reagent were accurately measured out into a series of test-tubes, and 0, 1, 2, 3, &c., cubic centimetres of the trimethylamine were introduced. After standing, the crystals formed were removed by filtration through glass wool, and a measured quantity of the filtrate was titrated with thiosulphate, and the absorption determined by comparison with the blank experiment.

(*a*) Reagent KI : I : Aq. as 4 : 3 : 100 grams.

With this strength the absorption was always variable, and increased with the number of cubic centimetres of trimethylamine solution added.

Thus, on calculating the formula of the periodide from the absorption of iodine observed, it was found that with 2 c.c. of hydrochloride solution the formula indicated would contain I_7, while with 3 c.c. the formula would contain I_8. On analysis of the crystals separated, however, they gave the normal result NMe_3HI_5. These results pointed to the precipitation of iodine by the aqueous solution employed, but no better results were obtained when the requisite volume of the hydrochloride was first evaporated to dryness before addition of the reagent.

(*b*) Reagent KI : I : Aq. as 4 : 1·5 : 100 grams.

With this strength precipitation was not complete. The absorption pointed to a formula containing only I_2, whereas the crystals obtained had the normal composition. The filtrate on treatment with a stronger reagent gave a further precipitation of crystals. In the course of a large number of experiments we were unable to discover the reason for the variations observed, and were consequently obliged to abandon the method.

Trimethylamine in Blood

Dessaignes[1] in 1856 demonstrated the presence of trimethylamine in calf's blood. The blood, which showed no signs of decomposition, was acidified, and twelve hours after its removal the clot was separated by pressing out the liquor. The serum obtained was distilled with lime. From the distillate of twelve litres of blood three kinds of platinum crystals were obtained, one of which Dessaignes proved to be trimethylamine platinochloride. One of us[2] first demonstrated the presence of trimethylamine as a normal constituent of human blood, and, using the method of de Filippi, found that in the healthy subject the amount per cent. varies between 0·0018 and 0·0011. It was found that in cases of uraemia there was a marked increase in the percentage of trimethylamine, and that this body is most probably responsible for the urinous smell of the breath of uraemic patients. In all these experiments the blood was treated in the same drastic method that was used by Filippi in his urinary determinations, and it was by no means certain that the trimethylamine might not have originated from the decomposition of lecithin phosphatides by means of the strong alkali added to the blood.

1. Dessaignes, *J. Phar.* (3) XXXII. 43.
2. Golla, *loc. cit.*

In 1906 Rzentowski attempted to determine the potential alkalinity of the blood by an estimation of the ammonia given up on boiling the blood in vacuo together with an ammonium salt. Though this failed as a method for determining alkalinity, his results showed that the potential alkalinity of the blood is abundantly sufficient to expel all the trimethylamine that the use of Filippi's method had shown us to be likely to be contained in the blood. It is, therefore, only necessary to boil the blood or serum in a vacuum at room temperature, and collect the distillate by passing it through a flask containing hydrochloric acid, which is surrounded by a freezing mixture. The trimethylamine and the ammonia are expelled by the potential alkalinity of the blood, and the distillation is proceeded with until the blood has been evaporated to dryness. Inasmuch as nothing is added to the blood, and it is never raised above the temperature of 20° C., this procedure cannot be held to have brought about the decomposition of lecithin or choline.[1] This method, though excellent for the identification of trimethylamine from the distillation of such small amounts of blood as 20 c.c., offers insuperable difficulties to the quantitative estimation of trimethylamine in larger amounts of blood, inasmuch as the frothing cannot be controlled. The proof that trimethylamine is given off under these conditions rests on microchemical tests, the validity of which we have already discussed, and the invariable production of the smell of trimethylamine on treatment of the alcoholic extract of the evaporated distillate with alkali. In our procedure the hydrochloric acid from the receiving flask is evaporated to dryness on a water bath, and the dry distillate again taken up with alcohol and evaporated. The residue is then examined by Florence's test, with Kraut's reagent, and another portion treated with alkali, and the characteristic smell of trimethylamine recognised. Using this method we have never failed to demonstrate the presence of trimethylamine in samples of fresh human blood and serum, of which over twenty have been examined. We have also shown the presence of trimethylamine in the blood of the cat, guinea-pig, ox, and the serum of the horse.

An attempt to estimate the trimethylamine of blood by a modification of this method was made. A litre of ox blood was treated with three times its volume of absolute alcohol, and filtered from the coagulated albumen. The filtrate was acidified with hydrochloric acid, so as just to redden litmus, and was evaporated to dryness at 37° C. The residue was

1. See further, p. 321.

taken up with alcohol, and the alcoholic extract again evaporated to dryness. The residue was then dissolved in 25 c.c. of water, to which 5 c.c. of decinormal sodium carbonate were added, and treated by the modification of de Filippi's method with sodium hypobromite already described. The acid neutralised by the distillate was titrated, and the results from three such experiments gave values of 0·001, 0·0015, and 0·0013 per cent. These results are in rough agreement with those published by one of us using the Filippi method for normal human blood.

An attempt was made to separate sufficient trimethylamine platinochloride from blood to allow of an analysis. For this purpose fifteen litres of ox blood were treated with alcohol, and distilled in the way described. The distillation, which was done in quantities of two litres of blood at a time, proved extremely tedious, and the amount of platinochloride obtained is in no way to be regarded as giving a quantitative estimation of the trimethylamine originally present in the blood. The platinochloride crystals purified by repeated extraction with alcohol gave on analysis the following figures:—

0·2590 grams of platinochloride gave 0·1345 CO_2 and 0·1050 H_2O.

Found C = 14·1; H = 4·5 per cent.

2 $NMe_3HCl.PtCl_4$ requires C = 13·7; H = 3·8 per cent.

The Presence of Trimethylamine in the Cerebrospinal Fluid

Through the kindness of Mr. McGavin, we have had exceptional opportunities for the examination of normal cerebrospinal fluid. Most previous observations on the chemistry of the cerebrospinal fluid have been conducted on fluid obtained by lumbar puncture from cases suffering from nervous disorders, or on post-mortem fluid, as the operation of lumbar puncture in the sound subject would have been unjustifiable. Mr. McGavin, however, allowed us to have the cerebrospinal fluid withdrawn from his numerous cases of lumbar anaesthesia, the custom being to withdraw a few cubic centimetres of fluid before introducing the stovaine into the lumbosacral puncture. By saving up this fluid, which was all obtained from cases suffering only from surgical disorders, relatively large amounts of pooled fluid were obtained, and a much larger material afforded to work upon than has been used by most other observers. Dr. Firth, from the Victoria Hospital for Children, and Dr. Eve, of Hull, also very kindly supplied us with fluid from cases of meningitis. With the fluid obtained from the clinique of Mr. McGavin we have been able

to make some thirty experiments with quantities of fluid varying from ten to fifty cubic centimetres. In every case examined we have found trimethylamine to be present and choline to be absent. Trimethylamine must then be regarded as a normal constituent of cerebrospinal fluid.

Method of Experiment:—The method employed was again that of distillation in a vacuum, but to avoid as far as possible the objection that any trimethylamine found in the distillate might have resulted from the decomposition of lecithin or choline—a point which will be discussed in the sequel—the distillation was conducted in a vacuum at a room temperature which varied between 15° and 20° C. The normal fluid being alkaline, nothing whatever was added to it, and the distillation was continued till only the dry residue was left in the flask. In a number of cases the proteid was first precipitated with carefully neutralised absolute alcohol, but in the majority the crude fluid was distilled. The volatile alkali was collected in well-cooled hydrochloric acid, which was afterwards washed out, evaporated to dryness at a gentle heat, the residue extracted with alcohol, and drops of the concentrated alcoholic extract allowed to evaporate on a microscopic slide. The dry residue was tested with the periodide reagent as previously described. When only small quantities of trimethylamine are present the recognition of the crystals requires considerable skill and practice, and it is, of course, highly desirable so to manipulate the alcoholic extract that the whole extracted matter is contained in the last one or two drops finally evaporated on the slide. If any trimethylamine is present a cloud of minute black globules is at once seen under the microscope, apparently streaming from the dry crystalline masses treated with the reagent. After a time these globules gradually coalesce, become elongated and crystalline. The neighbourhood of air bubbles under the cover-slip should be carefully examined as these seem to collect the droplets and crystals. The crystals formed seemed to be of a more permanent character than those described by Rosenheim with choline, and do not very readily re-form droplets.

Among experiments carried out in this way the following may be cited:—

(1) Jan. 17th, 1910. 47 c.c. of normal cerebrospinal fluid were received from Mr. McGavin. This was distilled in a vacuum at 20° C. and when two-thirds had passed over the distillate was evaporated, extracted with alcohol, and the alcohol extract converted to the platinum salt. Portions of this examined under the microscope showed on treatment with the periodide reagent clouds of droplets streaming out of the crystalline masses. These gradually elongated and crystallised in minute rod-like crystals exactly like the crystals figured by Rosenheim as choline periodide. Some of the larger droplets, however, showed a tendency to crystallise into

irregular, somewhat hexagonal formed crystals. On continuing the distillation of the fluid remaining in the flask to dryness and collecting the distillate in fresh acid, a considerable quantity of the platinum salt was obtained, each portion of which gave, with the periodide, a large number of minute rod-like crystals which are shown in the photograph (Fig. 3). The alcoholic extract of the residue remaining in the flask was repeatedly tested with the periodide reagent, but no indication of droplets or crystals could be observed.

(2) Jan. 1st, 1910. 35 c.c. of normal cerebrospinal fluid was distilled to dryness at 20° C. The evaporated distillate was taken up with alcohol, the extract allowed to dry up on a slide, and the residue treated with periodide reagent. In each case minute needles were seen under the microscope. The residue in the flask was extracted with alcohol, but the extraction on evaporation and treatment with periodide gave no crystals.

(3) Dec. 18th, 1909. 11 c.c. of normal cerebrospinal fluid treated in the same way showed many droplets some of which took on an elongated form, but no definite crystals were observed. This and similar experiments with small quantities would seem to show that this quantity of fluid was too small to give definite results.

Considering the numerous experiments that have been carried out with cerebrospinal fluid with a view to demonstrating the presence or absence of choline in it, it is a little surprising that with the exception of Gumprecht[1] no previous observes have recorded the presence of any base giving reactions similar to choline in fluids obtained from the normal animal, or in those suffering from diseases other than nervous. Halliburton[2] regards the base found by Gumprecht as choline, but Kaufmann,[3] who worked up a litre of cerebrospinal fluid, obtained chiefly from cases of paralysis, isolated a base which he regarded as certainly not choline. His method of experiment and conclusions have, however, been called in question by Donath,[4] who, with Halliburton, regards the presence of choline in cerebrospinal fluid as an accompaniment of degenerative nervous disease.

The explanation probably lies in the method of treatment of the fluid used by nearly all observers. Following the original method of Mott and Halliburton, the fluid is precipitated with alcohol and evaporated to dryness at 40° C. The alkalinity present would in these circumstances be sufficient to drive off any trimethylamine present, more especially if the process is repeated several times. The following experiment illustrates this:—

(4) 48 c.c. of normal cerebrospinal fluid was divided into two equal parts. One was made just acid with hydrochloric acid, and evaporated to dryness. The residue gave crystals with the periodide reagent. The other half evaporated by itself gave no crystals.

1. Gumprecht, *Verh. d. Congress f. innere Med.*, Wiesbaden, 320, 1900.
2. *Croonian Lectures*, p. 20, 1901.
3. Kaufmann, *Neurol. Centralblatt*, VI, 260 (1908).
4. Donath, *Neurol. Centralblatt*, 964, (1908).

Kaufmann is the only worker who acidified the fluid before evaporation, and, in our opinion, he must have obtained trimethylamine hydrochloride. Relying upon the smell of the residue when treated with alkali he obtained a peculiar 'spermageruch,' and says that this could not be trimethylamine. But the smell of trimethylamine, when very much diluted, agrees with Kaufmann's description. We were invariably able to smell trimethylamine in our own residues, for example:—

(5) 20 c.c. of normal fluid was treated as described by Kaufmann. Portions treated on a slide with a drop of potash solution were stated by three observers to smell of trimethylamine. The bulk of the residue was extracted with alcohol, and converted to a platinum salt, and this, with periodide gave irregular crystals as well as needles.

According to our experience, laboratory alcohol is frequently acid, and sometimes fairly strongly so, and it seems not at all improbable that in some experiments in which choline was found by other observers sufficient acid was present in the alcohol to neutralise the alkalinity of the fluid,[1] and to have permitted crystals of trimethylamine periodide to be obtained which would be described as choline. This may account for the discrepancies in the observations of various writers with regard to the presence or absence of choline in the cerebrospinal fluid of epileptics.

We have, in addition, found that if the alcoholic extract of the evaporated fluid be examined directly with the periodide reagent as recommended by Rosenheim, the sensitiveness of the reaction is very greatly diminished, and a large amount of trimethylamine or choline must be present in order to obtain crystals. The black droplets always form, but crystallisation is inhibited by the sodium, potassium and ammonium salts probably present.[2] This is well illustrated by the following experiments:—

Two mixtures A and B were prepared containing respectively per 100 c.c. 1 gram of salts (NaCl and NH_4Cl in equal proportions), and of trimethylamine hydrochloride (A) contained 0·02 gram and (B) 0·002 gram. 1/20 c.c. of each of these was evaporated at a low temperature on a slide, and the results obtained on treatment with the reagent compared with similar slides containing the same amount of pure trimethylamine.

1. Especially when we consider the small amounts of fluid used by some observers. Thus Kajiura (*Quart. Jour. of Exper. Physiol.*, Vol. I, p. 293), in one set of experiments employed 2·5, 3, 5, and 10 c.c. of fluid respectively.

2. Bertheaume, C.R. (1910). CL. 1065, however, says that the solubility of the crystals is lowered by the presence of salts especially by ammonium chloride.

		Weight of NMe_3HCl mgms.		Weight of salts mgms.		Periodide reaction
I.	(*a*)	0·01	...	—	...	Large crystals typical cross-like feathery form —visible to the naked eye.
	(*b*)	0·01	...	5	...	Immediate formation of droplets. In 20 minutes no crystals; large droplets. In 30 minutes a few small isolated rod-like crystals—large droplets.
II.	(*a*)	0·001	...	—	...	Numerous rod-like crystals easily obtained in a few minutes.
	(*b*)	0·001	...	5	...	Many small black drops; after a long time a few isolated crystals were seen.

These results explain our own early failures when large amounts of cerebrospinal fluid (up to 50 c.c.), acidified with hydrochloric acid, were simply evaporated to dryness, and the alcoholic extract of the dry residue tested directly.

The Possibility of the Trimethylamine obtained in the Physiological Fluids arising from the Decomposition of Lecithin or Choline

Cerebrospinal Fluid:—Our results have been unfortunately confined to normal fluids, and we hope that our method of detecting trimethylamine will be applied to fluids from various degenerative nervous diseases. In spite of the low temperature at which we worked, and the fact that we added nothing to the cerebrospinal fluid distilled, the objection that the trimethylamine found might originate from the decomposition of lecithin or choline requires attention. With regard to lecithin, what little evidence we possess goes to show that lecithin itself is not present in cerebrospinal fluid. Zdarek,[1] who examined 300 c.c. of fluid from a meningocele, was not able to detect any. We ourselves made one experiment in this direction. 47 c.c. of fresh normal cerebrospinal fluid were mixed with alcohol, and allowed to dry up in a vacuum. The dry residue was then ground up, and repeatedly extracted with a mixture of ether and alcohol. This extract on evaporation yielded a small quantity of greasy residue, which was boiled with strong nitric acid and the clear solution tested with a very sensitive ammonium molybdate solution. No indication of the presence of a phosphate could be observed. Although lecithin when treated with acids and alkalies[2] is fairly easily decomposed with formation of choline, yet the proportion of volatile nitrogen found by Maclean[3] as the result of treating lecithin cadmium chloride with such a

1. Zdarek, *Zeit. physiol. Chem.*, XXXV, 201 (1902).
2. *Zeit. physiol. Chem.*, LV, 352, 1908.
3. Maclean, *Zeit. physiol. Chem.*, LV, 365, 1908.

powerful reagent as alcoholic baryta solution was extremely small. The possibility of choline yielding trimethylamine under the conditions employed by us is very unlikely on a consideration of the results of Gulewitsch,[1] who has shown that choline is comparatively stable when treated with dilute acids and alkalies. In order to ascertain more exactly the effect of our working conditions on choline, we carried out the following experiment:—

(*a*) Preparation of choline hydrochloride free from trimethylamine hydrochloride—200 c.c. of a 2 per cent. solution of Kahlbaum's choline hydrochloride were mixed with 50 c.c. of a N/10 sodium hydrate solution and the liquid distilled in a vacuum as usual. After one hour at 35° C. the distillation was stopped. The distillate, evaporated and treated in the ordinary manner, gave crystals of trimethylamine periodide and the characteristic smell on the addition of potash. The liquid remaining in the flask was further distilled, and the distillate collected in fresh hydrochloric acid. But in this case no crystals were obtained. It appears probable that the trimethylamine found existed as an impurity in the choline, and was not formed by decomposition of the choline under the conditions employed. The liquid whose volume was now about 80 c.c. was exactly neutralised with hydrochloric acid, and made up to 100 c.c.

(*b*) 2 c.c. of the above choline hydrochloride solution were mixed with 8 c.c. of a solution containing 0·08 gram of sodium chloride and 0·08 gram of ammonium chloride, and 10 c.c. of N/10 sodium carbonate solution were added. This artificial alkaline cerebrospinal fluid gave on distillation in the usual way no reactions indicative of trimethylamine.

(*c*) In another experiment 15 c.c. of normal cerebrospinal fluid were distilled to dryness at room temperature. The distillate yielded crystals of trimethylamine periodide. To the dry residue in the flask was now added 2 c.c. of the above-mentioned pure choline solution, and 13 c.c. of distilled water. The distillate obtained from this mixture gave none of the tests for trimethylamine.

It would appear, then, as a result of these observations that our methods could be employed to detect trimethylamine in cerebrospinal fluid even in the presence of choline.

Conclusion

It would appear from the evidence obtained that trimethylamine is to be regarded as a normal constituent of the blood and the cerebrospinal

1. *Zeit. physiol. Chem.*, XXXIV, 514.

fluid. The evidence is not so conclusive as regards urine, since here the employment of an alkali other than that normally present in blood and cerebrospinal fluid might be held to cause a decomposition of some precursor of a phosphatide nature.[1] It seems equally clear that choline is not present as a normal constituent of the blood and cerebrospinal fluid, and, in common with other observers, we have failed to detect it in urine.

The results obtained with microchemical tests for trimethylamine make it obvious that no method of testing for choline can be valid when the trimethylamine is not taken into account. In the methods of Mott and Halliburton the presence of alkali must have eliminated all the trimethylamine during the process of evaporation, and these observers now admit that they fail to find choline in cerebrospinal fluid taken from the living subject, even in cases of general paralysis.[2] The evaporation of an extract of blood or cerebrospinal fluid as practised by nearly all the other searchers after choline would tend to drive off the trimethylamine more or less completely. The fact that laboratory alcohol is frequently sufficiently acid to more than neutralise the small amount of cerebrospinal fluid used is, we suspect, a reason for the discordant results recorded in the observations of those who have looked for choline in physiological fluids, and also explains the invariable finding of choline in blood treated with acid by Claude and Blanchetière.[3] It is to be noted that a further source of error in attempts to bring about the periodide reaction with very small amounts of blood or cerebrospinal fluid lies in the diminution of the sensitiveness of the reagent due to the presence of neutral salts, a source of error obviated by our method of distillation.

1. *See* Erdmann. *J. Biol. Chem.*, VIII, 57-60, 1910.
2. *Croonian Lectures*, 1910.
3. *Journ. de Physiologie*, Vol. II, 1909.

FIG. 1.—Crystals of Trimethylamine periodide, × 40, from 0·2 miligram of the hydrochloride.

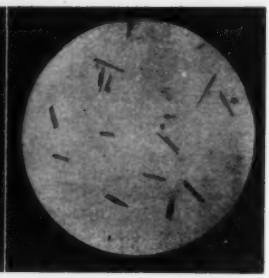

FIG. 2.—Crystals of Trimethylamine periodide, × 200, from less than 0·01 milligram of hydrochloride.

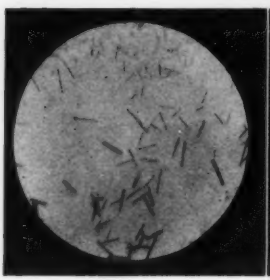

FIG. 3.—Crystals of Periodide. × 200, obtained from the distillate from normal cerebrospinal fluid.

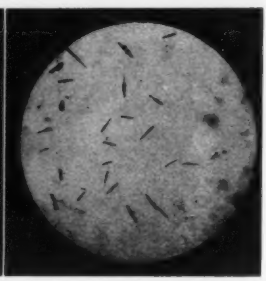

FIG. 4.—Crystals of Choline periodide, × 200.

STUDIES ON GLYCOSURIA: (*A*) CONSIDERATIONS OF MODES OF PRODUCTION OF GLYCOSURIA, AND THE CARBOHYDRATE, OXYGEN AND CARBON-DIOXIDE EQUILIBRIUM IN THE TISSUE-CELL. (*B*) RESPIRATION UNDER LOW OXYGEN PERCENTAGES AND UNDER HIGH CARBON-DIOXIDE PERCENTAGES IN THE RESPIRED AIR. (*C*) ANAESTHETIC GLYCOSURIA. (*D*) FEEDING EXPERIMENTS WITH PANCREAS AND INTESTINAL MUCOSA IN PANCREATIC GLYCOSURIA[1]

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Section A. Causation of Glycosuria and Carbohydrate Equilibrium

The separate means by which sugar in the urine, or glycosuria, can be induced are very numerous, and at first sight seem most dissimilar and heterogeneous, so that the study of this signal of a disordered carbohydrate balance becomes most perplexing from the chaos due to a multiplicity of apparently unconnected causes.

1. Many drugs produce glycosuria, amongst which may be particularly mentioned curare, morphine, strychnine, amyl-nitrite, and phloridzin, as well known and frequently corroborated; but a careful testing of drugs would probably reveal dozens of others.

2. All general anaesthetics induce glycosuria in varying degree, and this has been especially noted in the case of the commonly used anaesthetics, ether and chloroform; but it has also been found in nitrous ether anaesthesia.

3. Partial asphyxiation by coal gas causes glycosuria; as also does carbon-monoxide alone.

1. The expenses of this Research have been defrayed from a grant from the Government Grant Committee of the Royal Society.

4. Breathing an atmosphere rich in carbon-dioxide (over 13 per cent.) even in presence of an ample amount of oxygen for respiratory purposes has been shown by Edie[1] to produce glycosuria in marked degree.

5. Many nervous lesions and stimulations occasion glycosuria. The most noted of these is the piqûre of Claude Bernard, but many other procedures such as section of the vagi lead to glycosuria. It is noteworthy here that all those nervous lesions which produce glycosuria as a result, either cause a partial asphyxia from interference with the nervous respiratory mechanism or produce a local vaso-dilation and venous condition of a large area.

6. Glycosuria appears under conditions of senility of tissues, and with local gangrene.

7. Temporary ligation of the abdominal aorta in rabbits is followed by glycosuria.

8. Stimulation of certain parts by adrenalin, such as painting the pancreas or liver, leads to glycosuria.

9. The specially severe form of glycosuria known as pancreatic diabetes follows complete removal of the pancreas.

This long array of causes of glycosuria is by no means exhaustive of the sources narrated in the literature, nor does it enter into details; but it is a sufficiently heterogeneous list to demonstrate the apparent multiplicity of routes by which the symptom, or condition, of glycosuria may be reached.

The problem, apart altogether from its relationship to disease, is a most important one in bio-chemistry, because it lies deep at the root of the explanation of carbohydrate transmission and storage in plants and in animals.

In later years it has become clearly realized that protein, fat and carbohydrate are woven together into one organic whole in the bioplasmic molecule.

A great colloidal molecule or system of molecules is elaborated in the cell, with protein molecules probably playing the central rôle, but these are in a kind of molecular union with fats and carbohydrates. The fats and carbohydrates are by no means free, unless present in great excess, yet they are in such a condition that on a change of equilibrium they can be rapidly broken loose and set free as complete fatty or carbohydrate molecules. No fat may be visible to the microscope in a well-fed liver which contains a high percentage as shown by extraction with ether;

1. This Journal, Vol. I, p. 455, 1906.

but interfere with the nervous-supply, or blood-supply, of the organ, and fat droplets at once begin to show in the cells.[1] Similarly, it has been shown that a liver showing no obvious glycogen to miscroscopic observation in its living cells, on death of the bioplasm, and disruption of the bond of union between protein and carbohydrate may yield much glycogen, or if left to itself after death yields much sugar, far more than could be present in solution at the plasma percentage. Hence, living tissue contains within it in water-clear form, invisible to the microscope in discrete particulate phase, and lost to ordinary chemical tests, both fat and carbohydrate in forms insoluble in water when set free from the bioplasm. It is the sheer excess of glycogen or starch only which appears as cell granules. Such granule formation is a plethora of stored-up food after all the cell protein is saturated and satisfied. The working allowance, in generous amount, is in union with the protein, and may rise and fall during metabolism without any discrete phase with granules separating out.

Also in the blood plasma itself, the fat and carbohydrate are carried round in union to large extent with protein. A perfectly clear serum or plasma with no emulsion visible in it may contain as much as one per cent. of fat in various forms; an amount which could not be dissolved by the water and salts of the plasma, and which is kept in solution by the other organic constituents of the serum. Experimental evidence has also accumulated to show that all the sugar of the blood is carried by the plasma, the corpuscles containing practically none of it, and it has been proven that the sugar is held in a feeble kind of union most probably with the serum proteins.

In any case, it has been shown in this laboratory by Edie and Spence[2] that the amount of sugar dialysing away from the serum into saline is increased by the addition of carbon-dioxide or of anaesthetics to the serum. Edie has moreover shown, as stated, that breathing a high excess of carbon-dioxide leads even in presence of excess of oxygen to marked glycosuria.

These now well known facts as to union of protein in labile delicate equilibrium with fats and carbohydrates, have been recited because they appear to us to furnish a common basis on which many, if not all, of the common causes of glycosuria may be correlated and somewhat better understood.

1. Bainbridge and Leathes, *Bio-Chemical Journal*, Vol. II, p. 25, 1907.
2. This Journal, Vol. II, p. 34, 1907.

The secretion of sugar by the kidney may arise from one of two primary factors, viz., a variation in the amount of sugar in free solution in the plasma in the direction of increase so that there is hyperglycaemia: or, secondly, an increased sensitivity of the renal secreting epithelium, so that sugar secretion follows with a lower than normal amount of sugar in the blood or hypoglycaemia, this leading in turn to a disturbance of the carbohydrate metabolism of the tissue cells and diffusion out of sugar from the cell to restore the sugar content of the plasma, so giving rise to a continued secretion.

The glycosuria of phloridzin is the only form which appears to be accompanied by a hypoglycaemia, and hence to be due to heightened renal sensitivity; all other forms so far as they have been investigated in this regard are associated with hyperglycaemia, and hence with something which has its seat at the tissue cells, disturbing the equilibrium in such a direction that sugar dissociates off unless the osmotic pressure of sugar in the plasma is kept higher than the renal cells are able to withstand without secreting sugar out into the urine.

In attempting to obtain a connected picture of the various types of glycosuria and to correlate their very heterogeneous causes, we are hence driven back to study the conditions of equilibrium of carbohydrate metabolism in the cell and how this may be disturbed by various movements of the concentrations of the different soluble products concerned in the maintenance of equilibrium.

The sugar which under the abnormal conditions of glycosuria appears in the urine, would under normal conditions be oxidized to carbon-dioxide and water.

As a preliminary step to such oxidation the sugar molecule must first be built in as an intrinsic part of the bioplasm, disappearing meanwhile as free carbohydrate. During the time that the carbohydrate is in labile union with the bioplasmic molecule, oxygen is also taken up by the bioplasm from the plasma, and the carbohydrate molecule being rendered catalytically unstable by the action of the rest of the bioplasmic molecule, just as an enzyme renders its substrate unstable, union with the oxygen becomes possible and carbon-dioxide is given off.

Now in this delicately balanced equilibrium of (1) the catalyst or bioplasmic molecule, (2) the carbohydrate, (3) the oxygen and (4) the carbon-dioxide formed in the reaction, variations of the concentrations, or osmotic pressures, of any member of the system will lead to corresponding alterations in rate of oxidation.

If we take first the central figure of the reacting system, viz., the bioplasm, it is obvious that the power of this for acting as a receptor for carbohydrate will vary with the pressure of carbohydrate in the plasma and with the pressures of other dissolved substances which might possibly replace the carbohydrate in the same position (or vacant niche) in the bioplasmic molecule. If, for example, the molecule of an anaesthetic has a stronger affinity for the bioplasm than the carbohydrate, then dependent upon the concentration of the anaesthetic in the plasma, a certain amount of anaesthetic will enter into union with the bioplasm and to that extent the bioplasm will be placed *hors de combat* for oxidation of carbohydrate. Thus part of the glycosuria of anaesthesia may arise in this way from direct interference with the bioplasm and so preventing it from acting as an energy machine for oxidizing the carbohydrate.

Next we may take the concentration of the substrate itself, namely, the carbohydrate, in the plasma. Here it is obvious that other things being equal the higher the pressure of carbohydrate in the plasma, the more will pass into union with the bioplasm, and hence be in a position for chemical manipulation. But a limit soon will be reached if more and more is poured in; as, for example, from very excessive carbohydrate alimentation or intravenous injection. Part of this excess is dealt with by being converted into higher carbohydrates and fats for reserve food-storage by certain of the tissue cells, and, as we have seen, all cells have wide limits of quite invisible storage before visible droplets or granules of fat or glycogen appear. But if a certain upper limit is surpassed we reach 'alimentary glycosuria.'

The third factor in the equilibrium is the osmotic pressure of the dissolved oxygen, and this might seem at first sight the most important factor, but experimentally it is found that here there is a very large factor of safety, before the ordinary oxidation processes of the cell suffer from lack of oxygen.

Experiments on the lowest amount of oxygen necessary to support life have shown that in some species of animals this can be done on as low as four to five per cent. of oxygen in the atmosphere instead of the normal twenty-one per cent. of atmospheric air.

We have specially tested whether sugar appears in the urine under such conditions where animals breathe for a prolonged period the lowest amount of oxygen compatible with life.

Our experiments have been made with cats, rats, and rabbits, and we have found that *provided the carbon-dioxide formed in respiration is not*

allowed to accumulate in the atmosphere breathed by the animals, there is neither glucose in the urine nor any of those other products of incomplete oxidation, such as oxalates, which have been mentioned by other observers.[1]

In the experiments which we have carried out, instead of keeping the animals only for a period of an hour or so, as was formerly done,[2] the animals have been kept for over forty hours on five to six per cent. of oxygen, without any evil after effects, and *with absolutely no glycosuria.*

The fourth factor in the rate of carbohydrate oxidation, namely the osmotic pressure of the carbon-dioxide in the tissue-cell, is one which, in our opinion, has not yet received that attention which it deserves.

The form of chemical reaction most nearly allied to that of living cells is the enzymic action.

Here a colloidal catalyst, or chemical energy transformer, enters into a labile union with a substrate usually of an organic nature, and having united with it alters its chemical equilibrium, or balance of chemical potentials, in such a way that it is open to attack by a third body, such as an alkali or acid, and becomes hydrolysed, oxidised, or reduced, as the case may be. Now we know experimentally, and can easily understand on the general fundamental principles of chemical equilibrium, and kinetics of chemical reactions, that any accumulation of the products of reaction causing an increase in osmotic pressure and hence an increased chemical potential of these produced constituents, will act as a resistance upon the reaction, and with further increase finally bring it to a standstill with the different chemical potentials balanced against one another at certain definite osmotic pressures. The same thing has been shown conclusively for certain simple living organisms, such as the yeast plant, where the simpler constitution of the organism allows the reaction to be more definitely followed, and there is little doubt that this fundamental law of chemical kinetics holds for all living cells.

Returning now to the kinetic considerations of the production of glycosuria, as a result of hyperglycaemia, we readily see that the rate of utilization of sugar in the organism by oxidation, and hence the power of the various tissue cells to remove food sugar from the circulation and so prevent hyperglycaemia, may depend as much upon removal of carbon-dioxide as upon the presence of oxygen. In short, the reaction may be embarrassed and slowed as much from failure to remove carbon-dioxide

1. See Araki, *Zeitsch. f. physiol. Chemie*, Bd. XV, S. 335, 1891.
2. See v. Terray, *Arch. f. d. ges. Physiol.*, Bd. LXV, S. 393, 1897.

from the *rear* as from failure to supply oxygen in the *front* of the reaction.

Further, in the complex organism of the higher animals with its generous provision of a luxury of haemoglobin for carrying oxygen to the tissue, so that at any moment the oxygen pressure is at least four-fold what it needs to be for carrying on operations, the situation is actually reached that the rapid removal of carbon dioxide is even more exigent than the supply of oxygen.

For, although both arterial and venous blood carry more carbon-dioxide than oxygen, this is not the determining factor in respect to relative power of the blood as an oxygen-carrier and as a carbon-dioxide-carrier. The efficiency in this regard is determined by the variation in amount of dissolved gas, per unit variation in partial pressure of the gas. Now, round about the physiological working limits, on account of the chemical constitution of haemoglobin, there is a huge uptake or output of oxygen for a small variation of partial pressure of oxygen, which indicates the blood as a most excellent carrier of oxygen at these particular limits, keeping a very short *range* in the tissues of osmotic pressures of oxygen however the external partial pressure of oxygen may vary. There exists no such definite short range in the case of carbon-dioxide, for although various substances in the blood enter into labile union with carbon-dioxide and so act as carriers, there has none developed in the process of evolution with the definiteness of haemoglobin for oxygen.

Accordingly, if the partial pressure of carbon-dioxide in the lungs be varied by any procedures there is no very strong regulation of pressures, and the osmotic pressure of carbon-dioxide in the tissues will rise and fall fairly rapidly in a way which oxygen osmotic pressure never can do, until the very verge of asphyxiation is reached.

The same effects which follow variation of partial pressures in the lungs will follow upon impeded circulation, or stasis, in organs or gangrenous tissues. As the blood becomes more venous, the oxygen osmotic pressure only slightly decreases as it is pumped off from the reservoir in the haemoglobin, so that even in a very congested tissue the oxygen pressure may only be slightly below the normal, and oxidation processes are not embarrassed on this account. It is, however, quite otherwise with the carbon-dioxide pressures. In normal venous blood returning from an organ, practically all the special facilities possessed by proteins for holding carbon-dioxide in labile union are already used up. Blood cannot hold over fifty parts by volume of carbon-dioxide dissolved without the osmotic

pressure of dissolved carbon-dioxide mounting straight up in a purely physical fashion.

This fact has not hitherto been realised with any great clearness, but it is the key to the explanation of many important problems, including many of the forms of glycosuria with which we are here particularly concerned.

One must always be careful to distinguish an asphyxia due to excess of carbon-dioxide, from an asphyxia due to deficiency of oxygen. The former sets in under chronic conditions before the latter, and alters in a gradual way the whole life of the cell; the latter does not set in until there is nearly complete deprival of oxygen, and then comes on in an acute way with rapid death.

Leaving the subject of glycosuria for a moment, we may perhaps be allowed to point out the important bearings of this point of view upon certain fundamental bio-chemical problems. There is, firstly, the chemical regulation of the rhythm of respiration following carbon-dioxide variations much more sensitively than the oxygen variations, as has been shown by Haldane and his co-workers. The bio-chemical basis for this is very clearly shown by the above reasoning. For any moderate variation in partial pressure of oxygen in the lungs, such, say, as a fall to 10 per cent. instead of 20 per cent., will practically not affect the chemical equilibria in the nerve cells of the medulla or any other tissues of the body; on the other hand, a doubling of the percentage of carbon-dioxide in the lungs will lead almost to a simple doubling of the carbon-dioxide pressure in the medullary cells and elsewhere. Such a doubling must affect seriously the whole life-processes of the cells, for oxidation cannot go on if the products of oxidation cannot be got rid of from the catalyst which is inducing the oxidation. There is thus, it will be seen, not the slightest need to invoke the aid of secondary products of incomplete oxidation, such as oxalates, lactates, etc.; such bodies are truly secondary, and to cite them as the causes of increased respiratory activity in defective oxidation is most assuredly putting the cart before the horse. At most, they can only be contributory as part of a vicious circle; but they are not primary products of the oxidation reaction which is being embarrassed. The carbon-dioxide, which is a product of the reaction, is the primary cause by its accumulation and heaped-up osmotic pressure of the main oxidizing reaction being embarrassed. Other subsidiary possible reactions, of which carbon-dioxide is not a product, or is a less abundant product, thereupon commence to run, with increased comparative velocity, and here arise the pathological

products such as lactates, etc. But, remove the excess of carbon-dioxide, and all the rest falls away, as the reaction returns to its normal channels. Short of this excessive embarrassment by greatly increased carbon-dioxide pressure, there are no less interesting effects, especially to the pathologist, produced by chronic action of a slight excess of carbon-dioxide. Many of the chronic tissue changes induced by back-pressure and venous engorgement may be ascribed to increased pressure of carbon-dioxide. In work previously carried out by some of us upon the growth of artificially fertilized echinus eggs, we found that it was essential to guard against accumulation of carbon-dioxide, and that *small* amounts of carbon-dioxide artificially added gave rise to increased and pathological cell-divisions. From this point of view it is interesting to note that parts subject to chronic venous congestion are the seats of election for growth of tumours, both benign and malignant, in both sexes.

More than this inimical amount of carbon-dioxide stops cell-division in the rapidly growing organism entirely, as we found in our experiments upon echinus eggs; and it is similarly found, as, for example, by Waller in the case of muscle and nerve, that very minimal increases in carbon-dioxide stimulate to increased activity, but that more decreases and finally abolishes activity.

Returning now to the more immediate question of glycosuria and its production, we find experimentally, as mentioned above, that animals can be kept at the lowest amount of oxygen in the air compatible with life, for a period of nearly two days, without any appearance of sugar in the urine; if, on the other hand, they are kept for a like period of time in an artificial atmosphere containing at least 20 per cent. of oxygen, but in which by diminishing the nitrogen, etc., the amount of carbon-dioxide has been raised to over 14 per cent. by volume, then glycosuria is the result, varying in amount with the type of animal.

The explanation of this has been traced out above in that even at the lowest level there is enough oxygen for oxidation, and so long as the carbon-dioxide is removed oxidation can go on practically unembarrassed in the quiet animal, fresh oxygen in sufficient amount being brought round by means of the haemoglobin from an atmosphere containing about 5 per cent. of oxygen. If now, even with over 20 per cent. of oxygen available, there is also a partial pressure of 15 per cent. of carbon-dioxide in the cell then this almost completely locks the carbon-dioxide arising from oxidation into the cell catalyst, and although there is abundance of oxygen, oxidation of carbohydrate must cease.

In addition to this action there is probably also the purely physico-chemical effect seen in the experiments of Edie and Spence, whereby the carbon-dioxide simply detaches carbohydrate from protein, probably taking its place by direct substitution.

These experimental findings of the action of carbon-dioxide in producing glycosuria, and the physical chemistry underlying them, lead us onward to a common understanding of many of the forms of glycosuria due to drugs, anaesthetics, and interferences with nervous mechanisms, or blood supply.

Taking first nervous disturbances, we find that the lesions are such as would lead in large areas to a venous condition with increased carbon-dioxide pressure. For example, the puncture of the fourth ventricle in Claude Bernard's experiment disturbs the vagus nucleus, and leads by inefficient ventilation of the lungs to a high level of carbon-dioxide pressure throughout the body. Again, the operation of double vagotomy leads to a similar result.[1]

Also in two experiments not hitherto recorded, carried out by Moore and MacLean, in Liverpool, upon previously anaesthetized animals decerebrated by Sherrington's method, it was found that although the administration of anaesthetic was only kept up until decerebration was completed, that is, for a period of under half an hour; yet glycosuria persisted until the cessation of renal secretion, three or four hours later. It is, of course, impossible to decide clearly whether the continued glycosuria was due to the preliminary short anaesthetization or whether decerebration alone is capable of causing glycosuria; but here, although spontaneous respiration persists, there is no doubt that the condition is more venous than in the intact animal.

The continued glycosuria defeated the object of the experiments, which was to test the effects of removal of the pancreas afterwards, followed by intravenous injection of pancreatic extract.

The glycosuria in rabbits following temporary occlusion of the abdominal aorta may also arise from increased carbon-dioxide pressure during the tissue asphyxiation. The diabetes of gangrene also follows a

1. The original view of Bernard as to the cause of puncture diabetes, was that it was due to increased blood flow through the liver. In more recent times it has been suggested that this and other forms of glycosuria, and associated hyperglycaemia are due to asphyxia. (See Araki, *Zeitsch. f. physiol. Chemie*, Bd. XV, S. 335, 1891, Macleod, *Amer. Jnl. of Physiology*, 1907 *et seq.*) The main factor advanced here by previous workers has, however, been *deficiency of oxygen*, and not *increase of carbon-dioxide*, this we think is shown to be wrong by our negative results with excessively low oxygen pressures and positive results with high carbon-dioxide pressures. We suspect that certain of these results may have been due to carbon-dioxide and anaesthetic variations unsuspected by the authors. (See pp. 337-350 of this paper).

highly venous tissue condition. Partial general asphyxiation also leads to glycosuria.

The leading drugs which have been shown to cause glycosuria all possess an action which from different causes leads to an accumulation of carbon-dioxide in the circulation. Thus, curare induces paralysis of the respiratory system. So does morphia, and an overdose of morphia will by embarrassing the respiration lead to venous congestion and enhanced carbon-dioxide pressure in the tissues. Other narcotics, such as sulphonal, trional, and veronal, tested in this laboratory, upon animals, even in considerable doses did not lead to glycosuria, but these hypnotics do not cause such a generally venous condition as morphia. Amyl-nitrite, another drug which induces glycosuria, probably owes its effect, in part at least, to the general vaso-depression and venous condition which it induces. General lowering of blood-pressure alone is not sufficient without venous engorgement, for glycosuria does not occur after suprarenal removal, although the arterial system is under very low pressure. There is, however, in suprarenal inadequacy none of that flushing which follows nitrite administration due to venous congestion with high carbon-dioxide pressure in the tissues.

The glycosuria accompanying general anaesthetization, is probably due in part to the direct physico-chemical action of the anaesthetic in detaching carbohydrate from the protein in the bioplasm, in part to the condition of partial asphyxiation which the anaesthetic induces, and in part to the anaesthetic itself interlocking in the cell catalyst and arresting chemical action just as would carbon-dioxide, which is itself, from and on account of this action, a great natural anaesthetic.

Most of the apparently disconnected causes of glycosuria can accordingly be shown to be connected with an increased pressure of carbon-dioxide in the tissues, or the presence of bodies such as anaesthetics which act similarly to carbon-dioxide.

In the section on respiration under low oxygen pressures, the work of Araki is referred to, in which this author introduces a similar common explanation of (*a*) glycosuria due to reduced oxygen, and (*b*) glycosuria due to nervous lesions and many different drugs. The essential difference between our view and this of Araki, more recently revived by Macleod, is that we attach chief importance to carbon-dioxide increase rather than oxygen diminution.

Whether there is any similar condition after pancreatic removal would be exceedingly difficult to determine, since anaesthetization alone

leads to glycosuria. In fact, all experimental procedures are hampered by this unfortunate condition, and it has wrecked more than one scheme which we have laid for practical work on glycosuria.

So little stress is laid upon it by other workers who have obtained marked glycosuria after other experimental procedures, such as intravenous injections, that we venture to think there is a danger point here concerning which experimenters should be warned. At the commencement of our work we did not intend to work upon anaesthetic glycosuria. We had the usual amount of Hospital Ward information that after a severe and prolonged surgical operation a patient's urine might contain a little sugar, especially after chloroform anaesthesia. But we certainly did not suspect that an animal's urine within an hour after the induction of ether anaesthesia might usually contain 4 or 5 per cent., and occasionally had as much as 11 per cent., and that chloroform anaesthesia gave similar results with somewhat lower figures.

Since we had found that carbon-dioxide, a weak acid, had the physico-chemical property of detaching sugar from protein, we were led to suppose that an increased acidosis or more acid condition of the blood, such as usually obtains in diabetes, might similarly detach sugar and be the root cause of the glycosuria. On starting to experiment we at first thought our conjecture was correct, for on adding intravenously dilute acid (acetic, or β-oxy-butyric) we promptly obtained with the urine a marked reduction. However, in the next experiment, and within half an hour or so from the commencement of the experiment, *and before any acid had been added*, the urine was loaded up with sugar. This onset of anaesthetic glycosuria had misled us in the earlier experiment, and it has proved our *bête noir* ever since in all our work on glycosuria. Ruschhaupt[1] appears to have been the first to notice that ether administration in rabbits led to glycosuria, and the subject was afterwards worked out in detail by Seelig,[2] who reached the following conclusions:—

1. Ether inhalation in strong dogs always causes glycosuria.

2. Glycosuria is present throughout anaesthesia, even if this is kept up for seven hours.

3. The glycosuria disappears rapidly, clearing up in three to four hours.

1. *Arch. f. expl. Path. u. Pharm.*, XLIV, p. 140.
2. *Zeitsch. f. inn. Medizin*, XXIV, p. 221, 1903.

4. The urine of strong animals contained as much as 8 to 10 per cent., there being a lower percentage in weak or poor animals.

5. There was apparently hyperglycaemia accompanying the glycosuria.

Seelig further found that when ether inhalation was accompanied by intravenous oxygen injection there was no glycosuria; but that after glycosuria had set in it could not be removed by oxygen injection. This result, which would evidently connect glycosuria of ether inhalation with oxygen deficiency, has recently been confirmed by Macleod. It is however, difficult to conceive how ether anaesthesia would lead to a lower oxygen pressure than 5 per cent. in the respired air, which we have found never to lead to glycosuria, and also in certain of our own experiments with ether inhalation, we performed positive ventilation of the lungs with air, and there was certainly no oxygen defect which respiration in increased amount could avoid. We are hence driven to assume that there must be some other explanation for the non-appearance of glycosuria when oxygen is administered intravenously. The explanation may possibly be a physico-chemical one, implying not that the anaesthetic glycosuria is due to oxygen deficiency in the ordinary sense of the word; but the high partial pressure of oxygen produced by the venous administration may restore the balance so that the tendency to glycosuria, induced by such factors as ether pressure and carbon-dioxide pressure may be neutralized. There is, in fact, a combat between carbohydrate and oxygen on the one side, and ether and carbon-dioxide on the other for the valencies of the biogen molecule. If now oxygen pressure be heaped up, the scale may be turned away from glycosuria. That mere defect of oxygen alone can ever produce glycosuria is impossible, as far as we can see from our respiration experiments with low oxygen pressures.

Section (B).—Experiments on the Results of Respiration at (*a*) low Oxygen Partial Pressures, and (*b*) high Carbon-dioxide Partial Pressures

A very large number of observers have studied the effects of respiration under low oxygen pressures from Mayow and Lavoisier onwards, and the greater number by far of these make no reference either to the presence of reducing substances in the urine or the presence of such substances as show incomplete oxidation, such as lactates and oxalates.

The few earlier observers who studied the condition, as to glucose,

of the blood and urine after partial asphyxia always worked upon animals breathing in a confined space under such conditions that oxygen partial pressure fell and carbon-dioxide partial pressure rose simultaneously, and yet they all with one accord state that the glycosuria or hyperglycaemia is due to lack of oxygen, disregarding the other factor altogether.

This view is persisted in, as has already been indicated, down to the present day, no all-round consideration having been accorded to all the factors concerned in the equilibrium and kinetics of the oxidizing reaction in the tissues.

Alvaro Reynoso,[1] as far as we have been able to trace the matter, was the first to find glycosuria after partial asphyxiation, then Claude Bernard[2] proved that prolonged partial asphyxiation removed the glycogen of the liver, the removal being accompanied by glycosuria. Dastre[3] next investigated the matter, going into it more quantitatively. He found that an animal (dog) breathing free air had 0·128 per cent. of sugar in its blood; but when it breathed and re-breathed air for some time from a confined space the percentage in the blood rose to 0·253 per cent. The change was ascribed to lack of oxygen, but obviously the animal would at the same time be breathing a high percentage of carbon-dioxide, as in certain of our experiments recorded below.

Schiff[4] showed that ligation of a limb caused glycosuria, and gave us an explanation that the coagulation of the blood gave rise to a ferment which increased the production of sugar in the liver. Lepine and Boulud[5] ascribe such glycosuria to the production of 'leucomaines,' which interfere with glycolysis.

In our own opinion, the experiments below recorded furnish a much simpler explanation than either, viz., that the high carbon-dioxide pressure caused by the ligaturing of the limb causes the direct production of free glucose in the limb-tissues.

The only author we have been able to find who obtained both sugar and lactates in the urine after respiration of low percentages of oxygen, in absence, *as he supposed*, of any increased amount of carbon-dioxide, was Araki;[6] another observer who found lactates and oxalates in the urine, after keeping animals in a cage which was being rapidly ventilated by a mixture of gases containing a very low percentage of oxygen, and no carbon-dioxide, was Paul v. Terray,[7] but this observer found no sugar in

1, 2, 3. Cited from Dastre, *Comptes rendus*, LXXXIX, p. 669.
4, 5. Lepine and Boulud. *Comptes rendus*, CXXXIV, p. 582.
6, 7. *Loc. cit.*

the urine from his animals. It must be mentioned, however, that v. Terray's animals were treated for a much shorter period than those of Araki, so that sugar may not have had time to develop.

It is a most remarkable thing that though both these authors had their eyes open to the fact that the results of other workers were vitiated by the accumulation of carbon-dioxide at the same time that oxygen diminished, so that the two factors could not be dissociated, and although both of them arranged apparatus on their air current to deal with carbon-dioxide presence or accumulation, yet neither of them, as far as can be judged from their recorded papers, took any precaution to test whether their arrangements were efficient for the purpose intended.

That such arrangements were inefficient in point of fact is, we think, the explanation of the glycosuria obtained by Araki alone, and the presence of incompletely oxidized products such as oxalates and lactates obtained by both observers.

No analyses are given by either author of the air actually being breathed by the animals; but there is no doubt that there would be an accumulation of carbon-dioxide in both cases, and more particularly in the method of Araki. For v. Terray only sent the air through the chamber once, while Araki established a circuit, the air being, as he supposed, deprived of its carbon-dioxide while outside the chamber, by means of bubbling through strong potash before being returned to the chamber.

It is for this reason, we believe, that Araki obtained glycosuria, while v. Terray, like ourselves, obtained none.

The only effectual way, as we learnt by experience, is to deprive the air of its carbon-dioxide *in the actual respiratory chamber itself*, by an enormous surface of a powder such as soda-lime. Placing even a very large surface of strong caustic potash solution in the chamber itself is quite ineffectual, and merely bubbling the air through wash-bottles of strong caustic as it goes round in a pumped circuit is still worse. We have no hesitation from our own experience in stating that the air in the chamber used by Araki must have contained a high percentage of carbon-dioxide.

The method of placing a large tray or gallery of soda lime in the respiratory chamber has, moreover, the advantage in respiratory work, that rapid circulation is not required, with all its inherent difficulties, and the analytical errors which it introduces.

While in v. Terray's experiments, there would be no return of air containing carbon-dioxide to the respiration chamber, there is the serious

defect that the animal is breathing carbon-dioxide out into the chamber all the time, and usually such chambers are not large compared to the size of the animal inside. Hence, unless a veritable torrent of air is sent from the reservoir there must soon accumulate 2 or 3 per cent. of carbon-dioxide in the chamber. Even with a good water pump working full power, the rate of flow of air through the chamber would not keep the carbon-dioxide down below such a percentage.

In our own experiments, the percentage of carbon-dioxide was nearly always below 1 per cent., often considerably below that figure, and we have never found in the urine of animals, even when kept much longer than either Araki or v. Terray kept them, and at the lowest percentages of oxygen compatible with life (viz., 4 to 5 per cent. in rabbits), any abnormalities in the way of products of decreased oxidative activity, such as sugar or oxalates. When, however, the carbon-dioxide percentage exceeds about 13 per cent., even in presence of 20 per cent. of oxygen, there has invariably followed in our experiments marked glycosuria with a considerable percentage of sugar.

For comparison with the absence of sugar after low oxygen percentage alone, we have included certain of the experiments showing production of glycosuria by carbon-dioxide.

We also include an experiment in illustration of the above remarks to demonstrate how inefficient the placing of a strong solution of caustic potash in the chamber, or bubbling the chamber air through caustic potash, is in removing carbon-dioxide.

The apparatus used for these experiments was the same as that used before by Edie for the experiments on glycosuria induced by high carbon-dioxide percentages, and hence need only be briefly described.

The animal is enclosed, with food and with a tray of circular shape containing soda-lime, in a space shut off by a water-seal, and draws in its own oxygen, from a supply made from potassium permanganate by heating and kept in a large reservoir. As the oxygen cannot without special difficulty be made more than 95 to 96 per cent. pure, an aspirator is set flowing at a correspondingly slower rate, so as to prevent nitrogen accumulation and keep the oxygen percentage constant, or nearly so, throughout the experiment. Initially, either nitrogen is flushed through the chamber until the oxygen percentage falls to the proper limit, or the animal is allowed to automatically use up oxygen, and as the carbon-dioxide is taken up by the soda-lime, air is allowed to be sucked into the

chamber until the percentage is shown in a sample drawn off and analysed in a gas burette, to have reached the desired low level.

The apparatus is shown in section in the accompanying sketch, and a general photograph of it as fitted up is also shown.

In certain experiments, instead of allowing the animal to suck in oxygen, and adjusting for the nitrogen percentage in the oxygen supply by a correspondingly slower exhaust into an aspirator,[1] it was arranged

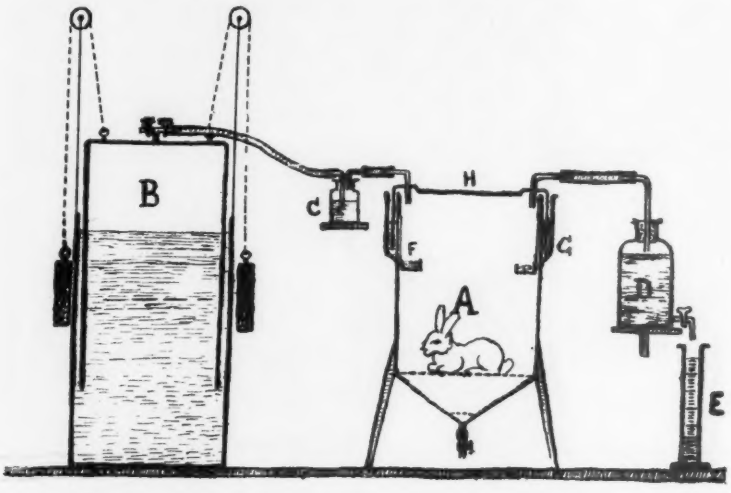

FIG. 1.—Sketch in section of respiratory chamber and accessory apparatus. Respiratory chamber, A; Oxygen reservoir, B; Wash bottle, C; Aspirator, D; Graduated vessel to measure outflow, E; Soda-lime holder, F; Water seal for lid, G; Glass pane for observing animal, H.

that there should be fed in an atmosphere of a known composition, and the gases of the respiration chamber were analysed at intervals during the experiment.

Experiment I.—Rabbit of 2·73 kilograms placed in cage with soda-lime at 9.40 a.m. The animal was allowed to suck in air automatically till percentage of oxygen fell to desired low percentage of about 6 per cent., which is near the lowest possible limit for a long experiment in the rabbit,

1. The mode of use of the aspirator is very simple. Supposing the oxygen fed in is 95 per cent. pure, and the internal air is to be kept adjusted at 7 per cent. of oxygen. Then every 100 c.c. of air sucked in introduces 5 c.c. of nitrogen which must be got rid of again; but if air inside has 93 per cent. of nitrogen, to get rid of 5 c.c. there must obviously be $5 \times \frac{100}{93} = 5{\cdot}4$ c.c. run off by means of aspirator or, roughly, aspirator must work at about $\frac{1}{20}$ of rate of oxygen current, which can readily be arranged by a little manipulation.

and can barely be supported by some animals. This animal, however, stood an amount of barely over 4 per cent. for a considerable period, that is, about one-fifth only of the normal atmospheric amount. It is to be remembered, however, that the animal was perfectly quiet in the cage, with no muscular work save that of breathing.

The results of analyses of the air of the respiratory chamber at various periods in the experiment were as follows:—

FIG. 2.—Photograph showing respiratory chamber and aspirating apparatus for adjusting percentage of oxygen in it. The lead pipe seen passing off at the left-hand border goes to the oxygen reservoir.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
9.40 a.m.	Commencement	Atmospheric
10.45 a.m.	1 hr. 5 min.	0·8 per cent. carbon dioxide 15·3 „ oxygen
1 p.m.	3 hrs. 20 min.	0·9 per cent. carbon dioxide 6·1 „ oxygen
1.30 p.m.	3 hrs. 50 min.	0·8 per cent. carbon dioxide 5·2 „ oxygen
3 p.m.	5 hrs. 20 min.	1·2 per cent. carbon dioxide 4·2 „ oxygen
4.45 p.m.	7 hrs. 5 min.	1·1 per cent. carbon dioxide 5·0 „ oxygen
6.30 p.m.	8 hrs. 50 min.	0·9 per cent. carbon dioxide 4·1 „ oxygen
8.30 p.m.	10 hrs. 50 min.	0·7 per cent. carbon dioxide 4·3 „ oxygen

The result of the experiment, through which the animal came quite well, was that 92 c.c. of urine were obtained at the end of the experiment, containing no sugar. Next morning a further 35 c.c. of urine were obtained also quite free from sugar. Oxalic acid was also absent from the urine. This experiment therefore clearly demonstrates that keeping the oxygen percentage at the lowest possible value compatible with life does not produce a trace of glycosuria, provided the carbon-dioxide percentage is not allowed to rise. In a subsequent experiment in which the carbon-dioxide pressure was allowed to mount up this same animal had glycosuria on a much higher percentage of oxygen. (See Experiment III.)

Experiment II.—The same animal and the same method used as in previous experiment.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10 a.m. ...	Commencement ...	Atmospheric
12 noon ...	2 hrs. ...	0·9 per cent. carbon dioxide 11·8 „ oxygen
2.15 p.m. ...	4 hrs. 15 min. ...	0·7 per cent. carbon dioxide 5·1 „ oxygen
3.45 p.m. ...	5 hrs. 45 min ...	1·0 per cent. carbon dioxide 4·3 „ oxygen
6.30 p.m. ...	8 hrs. 30 min. ...	0·5 per cent. carbon dioxide 4·0 „ oxygen
8.45 p.m. ...	10 hrs. 45 min. ...	0·4 per cent. carbon dioxide 3·8 „ oxygen
9.15 a.m. (next morning) ...	23 hrs. 15 min. ...	0·7 per cent. carbon dioxide 4·5 „ oxygen

At the end of the experiment 155 c.c. of urine were obtained free from sugar. The next quantity of urine obtained was also free from sugar and oxalic acid.

Experiment III.—In this third experiment the same rabbit was used as in Experiments I and II, but without soda-lime to keep down the carbon-dioxide in the respiration chamber, and at the same time the gaseous mixture admitted to the chamber was forced through and not drawn in automatically by the animal. Moreover, the reservoir contained as mixed gases nitrogen, oxygen, and carbon-dioxide, blended in such proportions that the air in the chamber being breathed by the animal was rich in carbon-dioxide, but also so rich in oxygen that the percentage of this gas never fell below that in ordinary atmospheric air. As a result we can see in this experiment the effects of carbon-dioxide alone, dissociated from any possible effects due to reduction of oxygen. The

results should be compared with those of Experiments I and II, where oxygen alone was down to the extreme limit of viability, but without much accumulation of carbon-dioxide.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10.15 a.m. ...	Commencement ...	—
10.45 a.m. ...	0 hrs. 30 min. ...	9·4 per cent. carbon dioxide 29·6 „ oxygen
12 noon ...	1 hr. 45 min. ...	14·7 per cent. carbon dioxide 26·9 „ oxygen
1.35 p.m. ...	3 hrs. 20 min. ...	22·0 per cent. carbon dioxide 22·5 „ oxygen
2.40 p.m. ...	4 hrs. 25 min. ...	26·5 per cent. carbon dioxide 21·6 „ oxygen
4.35 p.m. ...	6 hrs. 20 min. ...	26·4 per cent. carbon dioxide 22·7 „ oxygen
5.30 p.m. ...	7 hrs. 15 min. ...	27·9 per cent. carbon dioxide 21·4 „ oxygen

Experiment stopped at this period (7h. 15m.). No urine found in cage, but next morning 183 c.c. were found, giving a good reduction and showing on quantitative estimation 0·44 per cent. reckoned as dextrose. No oxalic acid found.

Experiment IV.—A different rabbit was used for this experiment weighing 1,726 grams. The animal was put in respiratory chamber with soda-lime. Nitrogen from a gas reservoir was then passed through, till the percentage of oxygen had fallen below 10 per cent., when connection was made with the oxygen reservoir, so that oxygen was sucked in as the expired carbon-dioxide was absorbed by the soda-lime. The aspirator bottle was adjusted so as to balance off small amount of nitrogen taken in with oxygen, and so keep percentage of oxygen in the respiratory chamber approximately the same at the low percentage.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
3.30 p.m. ...	Commencement ...	—
3·50 p.m. ...	0 hr. 20 min. ...	0·0 per cent. carbon dioxide 8·8 „ oxygen
8.50 p.m. ...	5 hrs. 20 min. ...	0·1 per cent. carbon dioxide 7·6 „ oxygen
8.55 a.m. ... (next morning)	17 hrs. 25 min. ...	0·2 per cent. carbon dioxide 7·8 „ oxygen
5 p.m. ...	25 hrs. 30 min. ...	0·0 per cent. carbon dioxide 7·6 „ oxygen
8.45 a.m. ... (second morning)	41 hrs. 15 min. ...	0·7 per cent. carbon dioxide 7·2 „ oxygen
3.45 p.m. ...	48 hrs. 15 min. ...	0·2 per cent. carbon dioxide 7·5 „ oxygen

The animal was not removed from the respiratory chamber during this period. It had moist bran in the chamber with it, which it ate at intervals. The urine was drawn off from the funnel-shaped bottom of the cage by putting on a little positive pressure inside and opening the clip on the rubber outflow tube.

In the first twenty-four hours 75 c.c. of urine were obtained, and in the next twenty-four hours 85 c.c. were found. Both lots were free from sugar, and also from acetone and oxalates. In the forty-eight hours the animal used up 50·45 litres of oxygen, and gave out 40·21 litres of carbon-dioxide, yielding a respiratory quotient of approximately 0·8.

The low value kept through this prolonged experiment for carbon-dioxide, and the constant low value for oxygen, make it, we think, a good test experiment for prolonged effects of low oxygen pressure. The animal appeared to suffer no serious injury from this prolonged stay in only about one-third of the atmospheric amount.

If deficiency of oxygen alone at such levels as are at all likely to obtain in diabetic piqûre, vagotomy, or morphia or similar drug poisoning, were at all likely to produce glycosuria, that condition ought to have appeared in this experiment, but there was not a trace of it actually found.

Experiment V.—In this experiment a cat of 2·73 kilos was used. The animal was put in the respiratory chamber with soda-lime in the tray. It was allowed to breathe air at first until the percentage of oxygen was reduced to about 11, after which the chamber was put in connection with the oxygen reservoir.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10.15 a.m.	Commencement	—
11.15 a.m.	1 hr.	0·2 per cent. carbon dioxide 15·2 „ oxygen
12.45 p.m.	2 hrs. 30 min.	0·3 per cent. carbon dioxide 9·3 „ oxygen
2.40 p.m.	4 hrs. 25 min.	No carbon dioxide 10·8 per cent. oxygen
3.50 p.m.	5 hrs. 35 min.	No carbon dioxide 9·7 per cent. oxygen

The experiment was stopped at this stage, as the animal had considerable dyspnoea. Next morning about 20 c.c. of urine were obtained free from sugar and oxalates.

In another experiment on a cat weighing 2·4 kilograms, carried out in

the same manner as above described, no urine was obtained, the animal becoming asphyxiated, although the percentage of oxygen throughout was much higher than that borne successfully by the rabbit.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10 a.m.	Commencement	—
11 a.m.	1 hr.	0·3 per cent. carbon dioxide 15·6 „ oxygen
12 noon	2 hrs.	0·3 per cent. carbon dioxide 10·9 „ oxygen
1.45 p.m.	3 hrs. 45 min.	No carbon dioxide 12·4 per cent. oxygen
3.30 p.m.	5 hrs. 30 min.	No carbon dioxide 11·4 per cent. oxygen

This experiment and the last show clearly that in the absence of carbon-dioxide cats become asphyxiated when the percentage of oxygen in the air breathed is reduced to about half the normal figure; whereas in the case of the rabbit existence is possible at a much lower limit of oxygen.

Experiment VI.—A rabbit of 1·7 kilograms was put in the respiratory chamber with soda-lime, and the experiment carried out exactly as above.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10.30 a.m.	Commencement	—
12.30 p.m.	2 hrs.	No carbon dioxide 14·5 per cent. oxygen
4.15 p.m.	5 hrs. 45 min.	No carbon dioxide 8·4 per cent. oxygen
5 p.m.	6 hrs. 30 min.	No carbon dioxide 4·6 per cent. oxygen
6.30 p.m.	8 hrs.	No carbon dioxide 3·0 per cent. oxygen
6.45 p.m.	8 hrs. 15 min.	No carbon dioxide 4·9 per cent. oxygen
9.30 a.m.	23 hrs.	No carbon dioxide 3·5 per cent. oxygen

The rabbit became asphyxiated during the night, the percentage of oxygen being then 3·5. At 6.30 p.m. the animal was exhibiting marked dyspnoea, the percentage of oxygen having dropped as low as 3, owing to an accidental constriction in one of the rubber connections between the chamber and the oxygen reservoir.

About 10 c.c. of urine were obtained by cutting open the rabbit's bladder. This urine was free from sugar.

Experiment VII.—A rabbit of 1·85 kilograms was used in this case, the details of the experiment being the same as before.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10 a.m.	Commencement	—
12.15 p.m.	2 hrs. 15 min.	No carbon dioxide 12·7 per cent. oxygen
1.30 p.m.	3 hrs. 30 min.	No carbon dioxide 9.7 per cent. oxygen
3.15 p.m.	5 hrs. 15 min.	No carbon dioxide 4·1 per cent. oxygen
3.45 p.m.	5 hrs. 45 min.	No carbon dioxide 13·3 per cent. oxygen
5.30 p.m.	7 hrs. 30 min.	No carbon dioxide 5·4 per cent. oxygen
7 p.m.	9 hrs.	No carbon dioxide 10·0 per cent. oxygen
9.30 a.m.	23 hrs. 30 min.	No carbon dioxide 7·3 per cent. oxygen

At the end of this experiment the rabbit was found asphyxiated. It had passed 100 c.c. of urine, free from sugar and oxalates. The urine in the animal's bladder was also free from sugar.

At 3.15 p.m., when the percentage of oxygen had dropped to 4·1, the animal showed dyspnoeic breathing, and was nearly asphyxiated. Hence it was thought advisable to increase the percentage of oxygen by washing out the chamber with a current of that gas until the animal had fully recovered. The experiment was then continued as usual until 6 p.m., when the animal was again partially asphyxiated.

Experiment VIII.—A rabbit weighing 1·76 kilograms was used for this experiment, which was carried out as already described.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
10.30 a.m.	Commencement	—
2.15 p.m.	3 hrs. 45 min.	No carbon dioxide 4·8 per cent. oxygen
2.45 p.m.	4 hrs. 15 min.	No carbon dioxide 10 per cent. oxygen
4.15 p.m.	5 hrs. 45 min.	No carbon dioxide 6·6 per cent. oxygen
6.30 p.m.	8 hrs.	No carbon dioxide 6·3 per cent. oxygen
11 a.m.	24 hrs. 30 min.	No carbon dioxide 8·3 per cent. oxygen

At the end of the experiment the rabbit was asphyxiated. It had passed about 150 c.c. of urine, containing neither sugar nor oxalates. At 2.15 p.m., the percentage of oxygen having dropped below 5, the animal began to be asphyxiated, for which reason it was relieved by a current of oxygen being rapidly passed through the chamber for a few minutes.

This was also done at 6.30 p.m.

In this experiment again we see that an animal which has been exposed to a percentage of oxygen sufficiently low to produce asphyxia cannot immediately afterwards breathe such a low percentage with safety.

Experiment IX.—Six rats were put in the respiratory chamber, and a mixture of carbon-dioxide and oxygen was used in order quickly to produce an atmosphere containing a high percentage of carbon-dioxide, with not less than the normal proportion of oxygen.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
11 a.m.	Commencement	—
11.25 a.m.	25 min.	9 per cent. carbon dioxide 24·2 „ oxygen
12 noon	1 hr.	20·3 per cent. carbon dioxide 23·4 „ oxygen
2.15 p.m.	3 hrs. 15 min.	21·4 per cent. carbon dioxide 25·3 „ oxygen
3.30 p.m.	4 hrs. 30 min.	21·1 per cent. carbon dioxide 27·3 „ oxygen

All of the rats were completely anaesthetised by the carbon-dioxide, and three succumbed during the course of the experiment.

Next morning 15 c.c. of urine were obtained from the surviving animals, and this contained 0·72 per cent. of sugar.

Experiment X.—In this experiment twelve rats were used in the manner described last.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
11.30 a.m.	Commencement	—
12.30 p.m.	1 hr.	14·9 per cent. carbon dioxide 29·1 „ oxygen
2.15 p.m.	2 hrs. 45 min.	18·2 per cent. carbon dioxide 29· „ oxygen
3.45 p.m.	4 hrs. 15 min.	28·2 per cent. carbon dioxide 23·3 „ oxygen
4.45 p.m.	5 hrs. 15 min.	25·6 per cent. carbon dioxide 23·4 „ oxygen

All the rats were anaesthetised in this experiment. At the end 10 c.c. of urine were obtained, free from sugar. This had probably been passed soon after the commencement of the experiment, when the animals became excited by the sudden inrush of carbon-dioxide. Next morning 14 c.c. of urine had been passed, containing 0·84 per cent. of sugar.

Experiment XI.—A cat weighing 2·6 kilograms was used in this case, and a mixture of carbon-dioxide and oxygen used to bring up the percentage of the former gas as rapidly as possible.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
11.20 a.m. ...	Commencement ...	—
12.10 p.m. ...	50 min. ...	3·3 per cent. carbon dioxide 22·5 ,, oxygen
2.45 p.m. ...	3 hrs. 25 min. ...	20·3 per cent. carbon dioxide 26·7 ,, oxygen
4.45 p.m. ...	5 hrs. 25 min. ...	20·5 per cent. carbon dioxide 24·8 ,, oxygen
6 p.m. ...	6 hrs. 40 min. ...	18·4 per cent. carbon dioxide 25·5 ,, oxygen

At this time 60 c.c. of urine were obtained, free from sugar. Next morning there were 62 c.c. containing 3·64 per cent. of sugar.

This shows that even in presence of 25 per cent. of oxygen a high proportion of carbon-dioxide in the air breathed produces very marked glycosuria in cats.

Experiment XII.—It has already been mentioned that we have found the attempt to absorb carbon-dioxide by means of potassium hydrate solution unsatisfactory, even when a large surface of solution was exposed to the products of respiration. This experiment shows how rapidly carbon-dioxide can accumulate in such a case.

A cat weighing 2·66 kilograms was put in the respiratory chamber, and in the bottom of the latter 350 c.c. of a 30 per cent. solution of potassium hydrate was placed, contained in a shallow flat-bottomed glass dish, about seven inches in diameter. The animal was allowed to breathe air until the percentage of oxygen in the chamber had fallen to about 16 by the accumulation of nitrogen, at which point the apparatus was put in connection with the oxygen reservoir, and the experiment continued

as in those cases where it was intended to keep the animal breathing a diminished proportion of oxygen, free from carbon-dioxide.

Clock time	Time Interval from beginning of experiment	Composition of chamber air
9.45 a.m. ...	Commencement ...	—
12 noon ...	2 hrs. 15 min. ...	10·5 per cent. carbon dioxide 8·4 „ oxygen
12.40 p.m. ...	2 hrs. 55 min. ...	12·4 per cent. carbon dioxide 6·6 „ oxygen

It will be seen that in three hours the carbon-dioxide produced by the animal accumulated in the chamber to over 12 per cent., this representing nearly the total quantity which an animal of this size would produce in that time. This shows that potassium hydrate, employed in the above-mentioned manner at least, is of practically no use as an absorbing agent for carbon-dioxide.

In the evening of the same day the cat was again put in the chamber, another freshly-made solution of potassium hydrate being used in the same way as before. In the course of two hours the carbon-dioxide had accumulated to 7 per cent., and the experiment was stopped. Next morning 120 c.c. of urine were obtained, with 1·1 per cent. of sugar.

It will be seen that cats are much more sensitive to diminution in the proportion of oxygen in the air breathed than rabbits, and at the same time a considerably higher percentage of sugar is generally obtained in the urine, when the animals are made to breathe air containing a high proportion of carbon-dioxide.

Section C. Anaesthetic Glycosuria

Some incidental account of the literature of glycosuria following anaesthesia by ether has already been given (p. 336). In the following brief outline of the literature of glycosuria due to chloroform anaesthesia we have borrowed a great deal from the valuable paper of W. H. Thompson[1] on 'Anaesthetics and Renal Activity.'

The presence of a reducing body in the urine after chloroform narcosis appears to have been noticed even in the earlier days of anaesthesia; but, whether the reducing body is actually glucose, in most cases would appear even now hardly to be settled.

1. *Brit. Med. Jour.*, p. 613, March, 1906.

Hegar and Kaltenbach[1] early experimented on the subject, and seem to have been the first to come to the conclusion that the reducing body was not glucose. Zweifel[2] found traces of chloroform in such post-anaesthetic urine, which of course would alone reduce Fehling's solution, but also confirmed the presence of another reducing substance, which he stated was not either uric acid or sugar. Kast[3] stated that the reducing property of the urine after chloroform anaesthesia was lost on removal of an organic chlorine compound. This author thought that carbohydrates are not increased, and that the reducing body is in all probability analogous to uro-chloralic acid (tri-chlor-ethyl-glycuronic acid) derived by oxidation from the chloroform and subsequent combination with the glycuronic acid. Nachod[4] attributes the reduction to an increase of kreatinin.

Thompson[5] found osazone crystals in five cases out of seven after chloroform anaesthesia; but states that in the majority of cases the crystals were unlike those of glucosazone in appearance, and gave melting points in impure conditions ranging about 130° C. In two cases there were undoubtedly glucosazone crystals present as well; the appearance was typical, and after fermentation with yeast they were no longer obtainable. The fermentation test gave unsatisfactory and inconclusive results in the other cases. No quantitative determinations of reducing power are recorded in this paper, which deals with many other aspects of renal activity after anaesthetics.

Thompson made examinations for osazone crystals after ten cases of ether inhalation in animals, and only obtained typical glucosazone crystals in two cases in which morphine had also been administered. Only four out of eight of the other animals gave crystals, and these were different from glucosazone crystals and resembled those seen after chloroform. The quantity, judged roughly, was smaller than after chloroform anaesthesia, and the frequency of occurrence also less in the case of ether. The substance yielding the crystals was probably the same in both cases.

1. *Virchow's Arch.*, Bd. XLIX, S. 43, 1869.
2. *Berl. klin. Wochensch.*, S. 245, 1874.
3. *Berl. klin. Wochensch.*, S. 377, 1888.
4. *Arch. f. klin. Chirurg.*, Bd. LI, S. 646, 1895.
5. *Loc. cit.*

In the experiments recorded below, the reducing substance was without doubt glucose, both after ether inhalation and chloroform inhalation. The substance was present in large amount and gave a quite typical reduction of Fehling altogether unlike creatinin, uric acid and similar bodies. The crystals, obtained in abundance, were undoubtedly glucosazone crystals; both by microscopical appearance, and by the fact that on careful purification by re-crystallization from hot alcohol and drying, they gave a melting point of 205° C. to 210° C., which agrees with that of glucosazone.

The animal used for these experiments was the dog, which received no other anaesthetic or drug than that being investigated; and the anaesthesia was kept complete during the experiment, at the expiration of which the animal was killed. As pointed out above, we came only incidentally to the study of anaesthetic glycosuria, our first objective being the effect of acidosis on production of glycosuria. Hence, in the earlier experiments, there are intravenous injections of dilute organic acids, as noted in the schedules (column three of each table); but the experiments are not invalid on this account, since, first, there is no rise in amount of glycosuria after the acid injection, showing that increased acidosis apparently has no effect upon glycosuria, and, secondly, exactly similar results are shown by the later experiments where intravenous injections of acid were not made, or were made much later in the experiment with no appreciable effect upon the glycosuria.

In case where the secretion of urine was scanty or failing, isotonic or hypotonic saline intravenous injections were made. It is remarkable how comparatively little of such intravenously injected saline reappears in the urine, although there is undoubtedly an appreciably increased diuresis. Incidentally we may mention that we often noticed very much increased secretion into intestine and peritoneal cavity after such saline injections.

The urine was collected by a cannula inserted into the bladder; the injections were made into the jugular vein.

The sugar determinations were made by precipitating the cuprous oxide in considerable excess of Fehling's solution, filtering into a Gooch crucible, and determining as cupric oxide by gravimetric analysis. This method we have found gives much more accurate results than a Fehling's volumetric analysis in any of the usual modifications, and when a large number of determinations are to be made, does not take a very great deal more labour.

Experiment I.—Dog of 5 kilograms. Ether anaesthesia. Animal under anaesthetic at 11-50 a.m.

Clock time	Time interval from start of experiment	Amounts injected of N/10 lactic acid +0·45 per cent. sodium chloride	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
a.m. p.m.	Minutes				
11.57—12.12	0—15	nil	0·7 c.c.	nil	nil
p.m. p.m.					
12.12—12.27	15—30	46 c.c.	1·15 c.c.	0·006	0·56
12.27—12.42	30—45	54·5 c.c.	1·2 c.c.	0·005	0·43
12.42—12.57	45—60	85·8 c.c.	1·8 c.c.	0·043	2·49
	h. m. h. m.				
12.57— 1.12	1 0 — 1 15	115 c.c.	2·6 c.c.	0·055	2·12
1.12— 1.27	1 15— 1 30	111 c.c.	4·0 c.c.	0·071	1·79
1.27— 1.42	1 30— 1 45	111 c.c.	7·3 c.c.	0·126	1·73
1.42— 1.57	1 45— 2 0	111·5 c.c.	5·8 c.c.	0·098	1·68
1.57— 2.6	2 0 — 2 9	65·5 c.c.	0·8 c.c.	0·015	1·93
2.7 — 2.22	2 10— 2 25	nil	1·6 c.c.	0·024	1·50
2.22— 2.37	2 25— 2 40	nil	7·2 c.c.	0·087	1·22
2.37— 2.52	2 40— 2 55	,,	5·8 c.c.	0·198	1·71
2.52— 3.7	2 55— 3 10	,,	5·8 c.c.		
3.7 — 3.22	3 10— 3 25	,,	3·8 c.c.	0·142	1·82
3.22— 3.37	3 25— 3 40	,,	4·0 c.c.		
3.37— 3.52	3 40— 3 55	,,	3·9 c.c.	0·111	1·85
3.52— 4.7	3 55— 4 10	,,	2·1 c.c.		
4.7 — 5.7	4 10— 5 10	,,	9·8 c.c.	0·068	0·70
5.7 — 6.7	5 10— 6 10	,,	7·1 c.c.	0·082	1·15

Totals and averages:	Total period	Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
	6 h. 10 m.	700 c.c.	76·4 c.c.	1·133 grams	1·49

NOTES.—At 2.6 p.m. flow of urine was stopping so injection of dilute lactic acid was discontinued. The animal later became oedematous and ascitic fluid was secreted in large amount. These injections of dilute acid fluid were not the cause of the glycosuria as shown by subsequent experiments.

Experiment II.—Dog of 4·43 kilograms. Ether anaesthesia. Cannulae in bladder and external jugular vein. Urine commenced to be collected at 12.45 p.m.

Clock time	Time interval from start of experiment	Amount injected of N/5 hydrochloric acid	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
p.m. p.m.	minutes				
12.45—1.15	0—30	nil	1·4 c.c.	nil	nil
1.15—1.45	30—60	50 c.c.	1·1 c.c.	nil	nil
	h. m. h. m.				
1.45—2.15	1 0 —1 30	50 c.c.	1·0 c.c.	0·018	1·83
2.15—2.45	1 30—2 0	50 c.c.	3·0 c.c.	0·009	0·33
2.45—3.15	2 0 —2 30	nil	1·6 c.c.	0·089	5·56
3.15—3.45	2 30—3 0	,,	2·0 c.c.	0·052	2·62
3.45—4.15	3 0 —3 30	,,	1·5 c.c.	0·004	0·25
4.15—4.45	3 30—4 0	,,	1·5 c.c.	0·002	0·13
4.45—5.15	4 0 —4 30	,,	2·4 c.c.	0·024	0·99
5.15—5.45	4 30—5 0	,,	2·3 c.c.	0·025	1·08
5.45—6.15	5 0 —5 30	,,	2·9 c.c.	0·034	1·18
6.15—6.45	5 30—6 0	50 c.c.	3·9 c.c.	0·027	0·70
6.45—7.15	6 0 —6 30	nil	4·4 c.c.	0·040	0·91
7.15—7.45	6 30—7 0	50 c.c.	4·0 c.c.	0·039	0·98
7.45—8.15	7 0 —7 30	30 c.c.	4·1 c.c.	0·014	0·34
Totals and averages:	Total period	Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
	7 h. 30 m.	280 c.c.	37·1 c.c.	0·377 grams	1·02

NOTES.—From 2.45 to 3.15 p.m. onwards, the animal required scarcely any anaesthetic to keep under deep anaesthesia. About 8 p.m. it was noticed that the stomach was enormously distended by gases. A post-mortem examination showed nothing abnormal in naked-eye appearances.

Experiment III.—Dog of 10·22 kilograms. Ether anaesthesia. Cannulae in bladder and external jugular vein. Fluid injected was N/2 acetic acid. Collection of urine commenced at 11.54 a.m.

Clock time	Time interval from start of experiment	Amount injected of N/2 acetic acid	Amount of urine excreted during each interval	Weight of dextrose excreted during each interval	Percentage of dextrose during each interval
a.m. p.m.	minutes				
11.54—12.24	0 30	nil	1·4 c.c.	nil	nil
p.m. p.m.					
12.24—12.54	30—60	100 c.c.	0·8 c.c.	slight trace	
	h. m. h. m.				
12.54— 1.24	1 0 — 1 30	nil	3·6 c.c.	Reduction increasing in amount all the time but precipitate of cuprous oxide is very fine and cannot be filtered off.	
1.24— 1.54	1 30— 2 0	,,	3·4 c.c.		
1.54— 2.24	2 0 — 2 30	,,	3·5 c.c.		
2.24— 2.54	2 30— 3 0	,,	4·2 c.c.		
2.54— 3.24	3 0 — 3 30	,,	7·4 c.c.	0·344	4·66
3.24— 3.54	3 30— 4 0	,,	7·0 c.c.	0·306	4·37
3.54— 4.24	4 0 — 4 30	,,	7·0 c.c.	0·303	4·33
4.24— 4.54	4 30— 5 0	,,	6·0 c.c.	0·291	4·85
4.54— 5.24	5 0 — 5 30	,,	4·3 c.c.	0·174	4·05
5.24— 5.54	5 30— 6 0	,,	4·0 c.c.	0·135	3·37
5.54— 6.24	6 0 — 6 30	,,	3·6 c.c.	0·118	3·27
6.24— 6.54	6 30— 7 0	,,	2·8 c.c.	0·045	1·60
6.54— 7.24	7 0 — 7 30	,,	1·9 c.c.	0·042	2·23
7.24— 7.54	7 30— 8 0	,,	4·6 c.c.	0·103	2,24
7.54— 8.24	8 0 — 8 30	,,	3·8 c.c.	0·074	1·94
8.24— 8.54	8 30— 9 0	,,	2·6 c.c.	0·056	2·14
8.54— 9.24	9 0 — 9 30	,,	2·6 c.c.	0·052	2·26
9.24— 9.54	9 30—10 0	,,	3·6 c.c.	0·074	2·07
9.54—10.24	10 0 —10 30	,,	2·6 c.c.	0·062	2·40
10.24—10.54	10 30—11 0	,,	2·8 c.c.	0·030	1·08
10.54—11.24	11 0 —11 30	,,	1·8 c.c.	0·037	2·06
11.24—11.54	11 30—12 0	,,	1·9 c.c.	0·029	1·54
p.m. a.m.					
11.54—12.24	12 0 —12 30	,,	2·8 c.c.	0·050	1·80
a.m. a.m.					
12.24—12.54	12 30—13 0	,,	4·6 c.c.	0·051	1·12
Totals and averages:	Total period	Total volume injected	Total urine excreted	Total weight of dextrose	Average percentage of dextrose
	13 hrs.	100 c.c.	94·6 c.c.	2·376 grams	2·51

NOTES.—The duration of ether anaesthesia here is for the remarkably long period of thirteen hours. It was noticed again that much less anaesthetic was required as the experiment progressed. The glycosuria persisted throughout the whole interval.

Experiment IV.—Dog. Ether anaesthesia. Cannulae in bladder and external jugular vein. Collection of urine commenced at 11.15 a.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
a.m. a.m.	minutes		grams	
11.15—11.45	0—30	3·0 c.c.	0·128	4·27
p m.				
11.45—12.15	30—60	2·0 c.c.	0·092	4·62
p.m.	h. m. h. m.			
12.15—12.45	1 0 —1 30	1·2 c.c.	0·029	2·45
12.45— 1.15	1 30—2 0	1·0 c.c.	0·035	3·51
1.15— 1.45	2 0 —2 30	0·2 c.c.	0·036	2·43
1.45— 2.15	2 30—3 0	1·3 c.c.		
Totals and averages	Total period	Total urine excreted	Total glucose excreted	Average percentage of dextrose in urine
	3 hours	8·7 c.c.	0·320 grams	3·70

NOTES.—The post mortem examination in this experiment revealed little of interest. The kidneys and liver appeared sclerosed, and the pancreas was very pale. The lungs were normal.

Experiment V.—Dog. Ether anaesthesia. Cannulae in bladder and external jugular vein. Commenced to collect urine at 1 p.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m. p.m.	h. m. h. m.		grams	
1.0 —1.30	0 0 —0 30	4 c.c.	0·078	1·94
1.30—2.0	0 30—1 0	8·2 c.c.	0·479	5·84
2.0 —2.30	1 0 —1 30	6·7 c.c.	0·764	11·40
2.30—3.0	1 30—2 0	6·2 c.c.	0·645	10·40
3.0 —3.30	2 0 —2 30	6·0 c.c.	0·697	11·62
3.30—4.0	2 30—3 0	6·2 c.c.	0·581	9·38
4.0 —4.30	3 0 —3 30	5·0 c.c.	0·429	8·59
4.30—5.0	3 30—4 0	5·5 c.c.	0·467	8·50
Totals and averages:	Total period	Urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	47·8 c.c.	4·140 grams	8·66

This experiment is remarkable for the high percentage of dextrose found in the urine throughout the whole period. The presence of dextrose was proved by examination of the osazone crystals prepared from the urine.

Experiment VI.—Dog of 5·01 kilograms. Chloroform anaesthesia. Cannula in bladder. Commenced to collect urine at 12 noon.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m.	h. m. h. m.		grams	
12.0 —12.30	0 0 —0 30	7 c.c.	0·071	1·01
p. m.				
12.30— 1.0	0 30—1 0	0·2 c.c.	0·519	3·53
1.0 — 1.30	1 0 —1 30	14·5 c.c.		
1.30— 2.0	1 30—2 0	11·4 c.c.	0·254	2·23
2.0 — 2.30	2 0 —2 30	5·2 c.c.	0·095	1·82
2.30— 3.0	2 30—3 0	5·4 c.c.	0·138	2·55
3.0 — 3.30	3 0 —3 30	11·1 c.c.	0·251	2·26
3.30— 4.0	3 30—4 0	6 c.c.	0·136	2·27
Totals and averages :	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	60·8 c.c.	1·464 grams	2·41

NOTES.—In this case again the presence of dextrose in the urine was proved by an examination of the osazone prepared from it. A post mortem examination showed that the animal's stomach was greatly distended with gas, which contained 29·7 per cent. of carbon-dioxide, and 1·1 per cent. of oxygen. Otherwise nothing abnormal was observed.

Experiment VII.—Dog of 8·62 kilograms. Ether anaesthesia. Cannula in bladder. Urine commenced to be collected at 12.15 p.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
p.m. p.m.	h. m. h. m.		grams	
12.15—12.45	0 0 —0 30	1·1 c.c.	—	—
12.45— 1.15	0 30—1 0	0·2 c.c.	0·034	7·55
1.15— 1.45	1 0 —1 30	0·2 c.c.		
1.45— 2.15	1 30—2 0	0·05 c.c.		
2.15— 2.45	2 0 —2 30	0		
2.45— 3.45	2 30—3 30	8·4 c.c.	0·362	4·31
3.45— 4.15	3 30—4 0	3·5 c.c.	0·206	5·90
Totals and averages :	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	4 hours	13·45 c.c.	0·602 grams	4·47

NOTES.—The excretion of urine was extremely slow at first in this experiment, consequently at 2.45 p.m. 200 c.c. of 0·5 per cent. sodium chloride solution were injected intravenously. No abnormal features were observed on a post mortem examination being made. The animal had been kept without food for a day previous to the commencement of the experiment.

Experiment VIII.—Dog of 9·65 kilograms. Ether anaesthesia. Cannula in bladder. Commenced to collect urine at 11.30 a.m.

Clock time	Interval from start	Amount of urine excreted during each interval	Weight of dextrose excreted in urine	Percentage of dextrose in urine
a.m. p.m.	h. m. h. m.		grams	
11.30—12.30 p.m.	0 0 —1 0	4·7 c.c.	0·052	1·11
12.30— 1.30	1 0 —2 0	4·6 c.c.	0·081	1·76
1.30— 2.30	2 0 —3 0	5·6 c.c.	0·087	1·56
2.30— 3.30	3 0 —4 0	4·7 c.c.	0·085	1·80
3.30— 4.30	4 0 —5 0	1·8 c.c.	0·056	3·11
4.30— 5.30	5 0 —6 0	4·0 c.c.	0·061	1·52
5.30— 6.0	6 0 —6 30	6·1 c.c.	0·085	1·39
6.0 — 6.30	6 30—7 0	4·4 c.c.	0·071	1·62
Totals and averages:	Total period	Total urine excreted	Total dextrose in urine	Average percentage of dextrose in urine
	7 hours	35·9 c.c.	0·578 grams	1·61

NOTES.—The dog used in this experiment had been fed principally on carbohydrates, but had received no food during the 24 hours preceding the experiment.

As the secretion of urine was very slow, 200 c.c. of water were run into the oesophagus at noon, and another 100 c.c. at 4.30 p.m.

Section D.—Feeding Experiments with Extracts of Pancreas, Intestinal Mucosa, and Liver in Pancreatic Glycosuria

In two successful experiments in which the pancreas had been removed in dogs under deep anaesthesia, the animals were given along with their food (*a*) pancreatic extract, (*b*) pancreatic extract to which extract of intestinal mucosa (duodenum) had been added, treated as in preparing secretin, and (*c*) a combination of extracts of pancreas, duodenum and liver. In the first of these two experiments the sugar output fell to zero, after a marked drop, so soon after administration of pancreas *plus* duodenum *plus* liver extract had been begun, as to appear a consequence of it. But next day sugar was present, although in diminished amount, and the third day the output of sugar was back to its original amount, and remained there in spite of administration of the combined extracts until the animal died three days later. No such result could be repeated with the second dog, and we cannot furnish any

explanation of this seemingly chance variation in sugar output, with entire absence for one whole period of twenty-four hours.[1]

In the first dog acetone and di-acetic acid were absent throughout the entire period; in the case of the second animal they were just as invariably present in large amount. Although the feeding results proved negative as regards any effect upon the glycosuria, the course of the diabetes and determinations not only of output of sugar, but that of urea, ammonia, acidity, and total organic acids of the urine, phosphates, etc., are sufficiently interesting to quote the two experiments in tabular form.

The amount of urine is given in c.c., and the total daily output of (*a*) sugar (gravimetric), (*b*) total urea (Dupre), (*c*) ammonia (Schlössing), (*d*) acidity and total organic acids (Edie & Whitley), (*e*) total phosphoric acid (Neumann), and total nitrogen (Kjeldahl) are given in grams.

1. Forsbach has recently recorded diminution of output of sugar after administration of a pancreatic hormone prepared by Zuelzer's method (*Deutsch. med. Wochensh.*, 1909).

TABLE I

Experiment 1.—Dog of 4·43 kilograms had pancreas completely removed under chloroform anaesthesia, weight of pancreas 14·74 grams. Glycosuria immediately followed on operation, from which the animal made a good recovery, but succumbed to diabetes and wasting after an interval of eighteen days.

Date 9 a.m.		Urine c.c.	S.G.	Total Sugar	Total Urea (Dupre)	Total NH_3	As HCl Total Acidity	As HCl Total organic acids	Total P_2O_5	Acetone	Di-acetic Acid	Total N (Kjeldahl)	Weight of dog in kilograms
Thurs., Dec.	7	80	1040	9·53	—	0·071	0·14016	0·226	—	0	0	—	4·43
Fri., ,,	8	365	1045	25·86	15·33	0·216	0·14921	0·418	1·102	0	0	6·26	—
Sat., ,,	9	285	1051	22·46	12·88	0·377	0·38691	0·214	0·756	0	0	7·25	4·20
Sun., ,,	10	305	1060	28·57	16·17	0·481	0·71693	0·283	1·381	0	0	7·57	—
Mon., ,,	11 Secretin	230	1056	20·74	12·54	0·374	0·48187	0·341	0·962	0	0	6·00	3·97
Tues., ,,	12	315	1058	29·06	18·74	0·405	0·70135	0·050	1·304	0	0	8·75	—
Wed., ,,	13	400	1056	33·41	22·60	0·634	0·87600	0·117	1·632	0	0	11·41	3·74
Thurs., ,,	14	510	1048	36·83	24·23	1·110	1·06106	1·113	1·945	0	0	11·71	—
Fri., ,,	15	350	1048	25·63	20·30	0·612	0·88148	0·473	1·521	0	0	9·47	3·63
Sat., ,,	16 S. + P.	460	1050	28·81	24·38	0·444	1·05105	0·829	2·186	0	0	10·90	—
Sun., ,,	17	315	1054	21·50	16·69	0·401	1·24100	0·533	1·658	0	0	8·36	—
Mon., ,,	18 Mon. night 2 doses	520	1049	38·57	23·40	0·647	0·72353	0·801	1·739	?	0	11·39	3·30
Tues., ,,	19	220	1052	8·45	18·26	0·456	0·64722	0·567	1·154	0	0	8·88	2·95
Wed., ,,	20	180	1048	0·85	15·21	0·482	0·56765	0·752	1·143	0	0	7·36	—
Thurs., ,,	21	215	1050	6·45	15·80	0·459	0·69058	0·662	1·503	0	0	7·54	3·07
Fri., ,,	22	555	1050	36·43	28·86	1·121	1·12227	1·450	2·384	0	0	14·40	3·07
Sat., ,,	23	445	1051	29·77	20·03	0·675	1·02650	0·942	1·983	0	0	10·52	3·07
Sun., ,,	24	370	1055	26·42	20·72	0·475	0·69686	0·160	1·623	0	0	9·49	2·73
Mon., ,,	25	50	1061	3·06	3·68	0·122	0·13505	0·026	**0.361**	0	0	1·70	Dead

TABLE II

Experiment II.—In a dog of 7·2 kilograms the pancreas was removed under deep chloroform anaesthesia, glycosuria followed, and the period of survival was fourteen days, details are given in Table II, as under :—

Date 9 a.m.	Urine c.c.	S.G.	Total Sugar	Total Urea (Dupre)	Total NH_3	As HCl Total acidity	As HCl Total organic acids	Total P_2O_5	Acetone	Di-acetic Acid	Total N (Kjeldahl)	Weight of dog in kilograms
Jan. 9	110	1057	0	9·9	0·163	0·108	0·453	0·890	0	0	4·93	—
„ 10	240	1064	0	34·32	0·595	0·856	0·964	1·514	0	0	17·04	7·27
Operation : complete removal of pancreas under deep anaesthesia. Weight of pancreas 21·15 grams. Weight of dog 7·27 kilograms												
„ 11	400	1040	13·31	15·60	0·359	1·146	0·252	1·773	0	0	8·56	7·05
„ 12	600	1074	53·99	44·10	0·889	1·730	1·486	2·934	Trace	0	20·60	7·27
„ 13	660	1057	55·64	39·29	0·867	1·157	1·080	2·202	Strong	Present	18·19	6·93
„ 14	490	1075	54·79	33·81	0·801	0·734	1·102	1·840	V. strong	Strong	15·85	6·70
„ 15	475	1076	51·18	34·91	1·205	0·843	1·555	2·019	Intense	V. strong	15·43	6·59
„ 16	580	1060	47·55	33·64	1·578	1·039	1·964	2·253	„	Intense	15·66	6·36
„ 17	460	1066	40·12	27·37	1·185	0·901	1·168	1·808	„	„	12·78	6·36
„ 18	510	1055	37·84	27·80	0·988	1·037	0·920	1·934	V. strong	V. strong	12·79	6·25
„ 19	420	1076	38·81	30·87	1·054	1·387	1·650	2·322	Intense	Strong	14·16	5·91
„ 20	295	1079	32·57	19·18	0·957	0·697	1·526	1·591	„	V. strong	8·58	5·80
„ 21	235	1083	24·93	21·50	0·763	0·690	0·989	1·214	„	Moderate	9·97	5·57
„ 22	275	1087	30·72	26·26	0·765	0·756	0·880	1·357	„	Slight	12·40	5·45
„ 23	220	1085	25·49	18·59	0·748	0·686	1·175	1·196	„	Trace	8·79	5·34
„ 24	510	1053	27·23	—	—	—	—	1·701	Weak	„	—	5·11
„ 25	500	1051	32·11	—	—	—	—	1·737	None	None	—	5·0

CREATIN AND CREATININ EXCRETION IN DIABETES MELLITUS

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Preliminary

In spite of a large amount of work by many investigators our knowledge of diabetes is limited, so limited indeed that it is still defined in terms of its most important clinical symptom:—'A chronic disease in which "grape-sugar" is excreted in the urine' (Von Noorden).

In many previous investigations the output of total nitrogen has been more or less thoroughly studied, but without any very important results being obtained. In the majority of instances the patients investigated were not confined strictly to one diet during the period of experiment, with the result that great variations in the output of nitrogen were observed. Further, in many cases the diet during the experimental period was—owing to the nature of the disease—very rich in protein. This abundance of protein in the diet naturally leads to a very marked output of nitrogen in the urine, to such a degree indeed that any slight variations which might have occurred from alterations in metabolic processes would be entirely obscured.

Von Noorden, although he recognises that this high elimination of nitrogen is mainly due to the amount of protein ingested, still affirms that a diabetic excretes more nitrogen than does a healthy man on the same diet.

Two causes are put forward to account for this increased excretion of nitrogen:—

(*a*) Loss of nitrogen from underfeeding; the diabetic being unable to utilise carbohydrate and thus to save protein, an increased catabolism of the latter takes place.

(*b*) Loss of nitrogen from toxogenous protein catabolism; the clinical picture of diabetic coma suggests that this may occur.

Lusk[1] and others have shown the output of nitrogen, particularly in relation to the sugar output, may yield results of great clinical (prognostic) importance. They have shown that after the removal of the pancreas in dogs—whether these be fasting or on a meat diet—there is a constant elimination of nitrogen (N) and dextrose (D) in the urine (Minkowski), and these two substances are always present in the same proportion every day; there are 2·8 grams D for 1 gram N—D : N : : 2·8 : 1. Since each gram of nitrogen in the urine represents a destruction of 6·25 grams of protein in the body, it is evident that 2·8 grams of dextrose may arise from 6·25 grams of protein, or protein yields 45 per cent. of dextrose. Lusk (*loc. cit.*) determined the amount of sugar phloridzinised dogs would produce when gelatin was supplied, and found a higher D : N ratio of 3·65 : 1, which represented a sugar production from protein of nearly 60 per cent. This higher ratio has been found in human diabetes when the patient is on a diet of protein and fat (Mandel and Lusk), and also in dogs when both pancreas and thyroid have been removed (Falta).

The variability of the D : N ratio in different animals and in the same animal under different circumstances cannot yet be definitely explained. Falta suggests that the extirpation of the pancreas and thyroid gland leaves the adrenals highly active, furnishing a secretion which tends to promote the formation of sugar. Mandel and Lusk assume the existence of two different chemical combinations of protein and sugar—an alpha-colloid dextrose representing the sugar in the 2·8 ratio, and a beta-colloid dextrose representing the additional percentage of the protein when the 3·65 ratio is present, the ratio depending on the combustion of the latter. The higher ratio has been termed the 'fatal ratio,' as it shows a complete intolerance for carbohydrates, a lower ratio indicates hope for the patient. It is recommended as a matter of routine in practice that the patient should be placed on a strict carbohydrate-free diet and on the second day the D : N ratio determined.

Cathcart[2] in a recent paper has, as the results of many experiments using diets of varied nature, put forward the hypothesis 'that carbohydrates are absolutely essential for intracellular synthetic processes in connection with protein metabolism.' In his investigations he used the output of creatin—not a normal constituent of healthy urine—as the index of protein catabolism. This substance was chosen because it is constantly present in the urine during starvation (Cathcart[3], Benedict[4]). Cathcart (*loc. cit.*) found that when the diet consisted only of carbohydrates or was one comparatively rich in carbohydrates, although it might be relatively

low in Calories, no creatin was ever found in the urine. He also found that, even when the feeding followed a period of starvation,—which had led to the appearance of creatin in the urine,—that on a carbohydrate diet the creatin immediately disappeared. On the other hand, if carbohydrates were completely absent from the diet or present only in very small quantity, even when the intake of Calories was abnormally high, creatin appeared in the urine; and, if the feeding followed a period of starvation, the output of creatin instead of diminishing, as when carbohydrates were used, increased in amount. As a result of these experiments the hypothesis above mentioned was formulated, the suggestion being that the presence of carbohydrates in proper form is essential before the anabolic processes in connection with the utilisation of protein in the body can take place. In support of his contention he brought forward much collateral evidence.

An experiment performed on myself at Dr. Cathcart's request has considerable significance in connection with this point. A strict carbohydrate and creatin-free diet was taken, consisting of 10 eggs, ½ lb. butter, and one diabetic loaf (6 oz.). The Caloric intake was ample. The figures obtained were as follows:—

Exam.	Amt. urine c.c	Sp. gr.	React.	Total Nitrogen	Creatinin	Creatinin	Creatin
1.	Sample	1018	Acid	1·3 per cent.	0·21 per cent.	0·21 per cent.	Nil
2.	1,400	1020	,,	13·6 grams	1·567 grams	1·5 grams	0·067 grams
3.	1,200	1018	,,	17·1 ,,	1·536 ,,	1·4 ,,	0·136 ,,
4.	1,150	1025	,,	17·0 ,,	1·9 ,,	1·9 ,,	Nil

The first examination represents the analysis of a sample of urine when on ordinary mixed diet.

The second and third are the results obtained while the special diet was being taken. As will be seen, creatin was found.

The fourth examination was made after the addition of carbohydrate to the diet. Creatin had disappeared.

Another exactly similar experiment carried out on Dr. Cathcart had the identical results.

Since diabetes is the disease in which carbohydrate metabolism is notoriously faulty (*vide supra*), it was thought that even on a creatin free diet creatin would be present in the urine of diabetics in large amount. This has proved to be the case. It was further thought that alterations in the output of creatin might prove of value from the prognostic standpoint; that a diminution in the output would indicate that the utilisation

of carbohydrate was improving, or, if the output increased, that the disease was increasing in severity. This expectation has not, however, been realised.

In the acute forms of diabetes mellitus there is complete loss of power to burn dextrose, and probably the tissues do not retain glycogen. Such an organism must exist at the expense of protein and fat as sources of its potential energy. But in diabetes a major portion of the ingested protein is convertible into sugar, which is carried away in the urine, and this may contain a large proportion of the potential energy of protein available for cell life. To compensate for this the protein metabolism increases (as much as three to five times); but fat metabolism remains the mainstay of the life of the diabetic as it does of the fasting individual. In diabetes the protein metabolism is abnormal, and conditions varying in severity also arise in which the end-products of fat metabolism, such as beta-oxybutyric acid, aceto-acetic acid and acetone, do not burn, but accumulate within the organism and are eliminated in the urine.

Voit believed that protein breaks up into a nitrogenous portion convertible into urea, and a non-nitrogenous portion which as sugar or fat can be used by the organism. Embden and others, however, have pointed out that the amino-acids are convertible into dextrose, and that they probably are the source of urinary sugar in diabetes. Pflüger, on the other hand, believed that the sugar came from fat.

The literature shows that a certain number of observations have been made in connection with the excretion of creatinin in diabetes. Creatin on the other hand, has received less attention. Indeed, the only reference found with regard to the latter in diabetes consists of the brief mention of two cases by Shaffer,[5] in both of which it was found.

Prior to the introduction in 1905 of Folin's method of estimating urinary creatinin and creatin many observers had worked at the subject. The results are fully discussed by recent writers: Von Noorden, Mellanby,[6] Shaffer (*loc. cit.*), and others. The latter gives an outline of the more important findings, and adds that these must be accepted with caution on account of the methods employed.

Von Noorden states that it was early recognised that the excretion of creatinin was increased in diabetes, up to 2 grams per diem; an increase said to be due to the condition of nitrogenous metabolism obtaining in diabetics; the patient eats a great deal of meat and possibly breaks down his own muscle substance. In the same section this writer, referring to Folin's paper,[7] in which this author demonstrated that the urinary

creatinin has both an endogenous and an exogenous origin, remarks 'that it would be extremely interesting to know whether or not the endogenous creatinin of diabetics ever shows considerable variations from the normal 0·4 gram—0·6 gram per diem.'

Bunge[8] states that the patient does not excrete more than a healthy man on a purely meat diet, viz., 2·163 gram per diem.

Folin (*loc. cit.*) first observed that the amount of creatinin excreted in the urine by a normal individual is quite independent of the amount of protein in the food, and of the total nitrogen in the urine; the amount excreted from day to day is practically constant for each individual under similar conditions of health and muscular activity. Shaffer (*loc. cit.*) has further found that the hourly excretion of creatinin is also a constant quantity, and is quite independent of the amount of urine passed, or the amount of protein ingested. Folin (*loc. cit.*) has also shown that the chief factor determining the amount of creatinin eliminated is the live body weight of the individual. Wolf and Shaffer[9] have confirmed a further statement made by Folin and Klercker that the excretion of creatinin is wholly unaffected by the ingestion of creatin.

On the other hand, the experiments of Noël Paton[10] and Cathcart[2] have shown that there is in all probability a very close connection between the output of creatinin and creatin. In one experiment of Cathcart's it was shown that with a rise in the output of creatin there was a marked fall in the output of creatinin. There is also a certain amount of evidence to show that under certain circumstances the ingestion of food containing creatin is followed by a rise in the output of creatinin. The following short experiment performed on myself bears on this point. A diet containing 480 grams meat was taken on the day succeeding a creatin-free diet. The figures were as follows:—

Exam.	Amt. urine	T. N.	Total Creatinin	Creatinin	Creatin	Remarks
	c.c.	grams	grams.	grams		
1.	1600	10·7	1·68	1·68	Nil	Creatin-free diet
2.	1500	15·7	2·347	2·17	0·177	Abundant flesh diet

As will be seen creatin was excreted on this abundant flesh diet, and this occurrence was associated with a rise in the total nitrogen and creatinin outputs.

It has already been mentioned that Folin (*loc. cit.*) believes that the amount of creatinin excreted in the urine is a constant quantity for the individual, and independent of the nitrogen intake, or of the total nitrogen passed in the urine.

The figures in the tables accompanying this paper also indicate a connection between the creatinin excretion and the nitrogen intake, and they further show that the output of creatinin in diabetics is by no means always greatly exaggerated, even when a highly nitrogenous diet is being taken.

Von Noorden gives conflicting figures when considering the effect of diet on the daily output of creatinin; with a mixed diet of 0·8 gram—1·2 gram per diem; with vegetable diet 0·61 gram—0·86 gram. He quotes Macleod and Long. The former obtained higher values:—with 'mixed meat diet' 2·098 grams, and with 'creatin-free' food 1·064 grams. The latter found 0·9 gram in vegetarians.

The dietaries of the patients examined for this paper were rarely excessive in flesh content, and in a large majority of the cases a creatin-free food was given, the persistence of creatin in the urine during these periods must have been due to causes other than the food ingested.

The earlier estimates of creatinin excretion during muscular work vary, and are probably of little value on account of the defective methods employed. Von Noorden states that excessive muscular work may cause creatin to appear in the urine. Cathcart, Kennaway, and Leathes[11] found that even severe work does not cause a rise in the urinary creatinin when the diet is ample. Shaffer's work[12] led him to express the opinion that the amount of muscular activity is in itself entirely without effect upon the amount of creatinin excreted.

Quite recently Graham Brown and Cathcart,[13] in a paper, in which the literature on this point is reviewed, found that stimulation of muscle might actually decrease the amount of total creatinin (creatinin + creatin) extracted. Mellanby (*loc. cit.*), on the other hand, states that work has no influence on the creatin content of muscle. Pekelharing and v. Hoogenhuyze[14] found that rigor mortis, heat rigor, and 'tonus' led to an increase of creatin in muscle.

So far as this investigation is concerned, the influence of muscular work on the urinary creatinin and creatin excretion may be ignored. The patients examined—with one exception—being all hospital in-patients either entirely at rest in bed or only doing the light work of the wards.

Creatin when present in a creatin-free diet is a product of pure endogenous origin, and is never found in the urine as an excretory product under normal conditions (Folin). It can, however, be readily caused to appear, its chief source of origin being the muscle tissues.

Voit believed that it was present in urine alkaline when passed (Von Noorden). This has been shown to be incorrect.[5]

The latest contribution to the literature on the subject of creatin excretion is an article by Noël Paton,[10] who shows that in the urine of birds creatin takes the place of creatinin, and is an end product of metabolism, and that increased creatin excretion indicates an increase of muscle catabolism.

The urinary creatin excretion has been examined in certain diseases. In nephritis it is said to be increased, and was at one time thought to accumulate in the blood and be a factor in uraemic poisoning (Von Noorden). This, however, has been denied, as it probably never occurs in sufficient quantities, and it is a less toxic substance than creatinin. Turner[15] refers to recent work by Donath, who records the results of his experiments into the convulsion-producing properties of the substances found in the blood and urine of epileptics. When injected into guinea-pigs and dogs creatin proved innocuous; creatinin, on the other hand, produced convulsions.

A sample of urine from a female patient, who died of diabetic coma, was examined, and the creatin value was distinctly high. Total creatinin 0·0448 gram per cent. Preformed creatinin 0·0224 gram per cent., and creatin 0·0224 gram per cent. It has also been noticed that in those cases in which the acetone and diacetic acid tests were most marked the creatin values were highest (e.g. Table VIII).

Mellanby (*loc. cit.*) tabulates the excretion of creatinin and creatin in various pathological conditions affecting the liver; he found that the output of creatinin in hepatic disease is markedly sub-normal on account, he believes, of depressed hepatic function, while in cancer of the liver creatin is excreted in large quantities; in cirrhosis and engorged livers on the other hand, creatin excretion is not affected. This writer is of opinion that the liver is intimately connected with the production of creatin and the excretion of creatinin. The creatin excretion in pathological states other than those already mentioned has been examined by other workers. Shaffer[5] mentions the following conditions, in all of which there is probably a breaking down of muscle tissue; acute fevers, the acute stages of exophthalmic goitre, tumour cachexia. The largest output found was in women during the first week post-partum. Finally, in the graver forms of blood diseases the creatinin values are low, and creatin has been found in lymphatic leucaemia (Shaffer) and myelogenic leucaemia (Von Noorden).

The following then appear to be the conditions under which creatin may occur in the urine as an excretory product. Starvation, excessive

muscular work on an insufficient diet, heart disease with failure of compensation, certain forms of liver and blood diseases, nephritis, Graves disease, fevers, tumour cachexia, epilepsy, and during the early days of the puerperium.

In the series of cases examined for this paper all these conditions may be excluded.

The methods employed for the various examinations connected with this paper were:—Total nitrogen (Kjeldahl), creatinin and creatin (Folin);[1] sugar (Pavy). In order to acquire familiarity with these methods three patients suffering from diabetes were taken. These were all undergoing hospital treatment and were improving. The figures are not tabulated, but it may be stated that creatin was always found in the urines, and that the total nitrogen and creatinin excretions were not high. An attempt was made—as will be noted in the various protocols—to test the various patients with a diet which was absolutely free from either creatinin or creatin. Such a diet, free from carbohydrate and yet palatable, was impossible. Accordingly, from time to time a diet was used which—although free from creatinin and creatin—was rich in carbohydrates. As such a diet is quite unsuited for a diabetic patient, the results obtained were, on the whole, very unsatisfactory. This diet consisted of:—eggs, milk, cream, oatmeal, cheese, butter, diabetic bread, water. The tables may now be appended.

TABLE I—Female aet. 53. Weight, 7 st. 10½ lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			grams.	grams.	grams.	grams.	grams.	
June 18...	7,360	1045	Acid	268	39·7	1·008	0·883	0·125	Ordinary mixed diet
22...	4,460	1033	Acid	85·6	24·9	1·517	0·624	0·893	Third day after diabetic diet begun
„ 25...	4,206	1034	Acid	91·0	23·9	1·345	0·715	0·630	„ „
„ 28	4,265	1040	Acid	125·4	24·3	1·531	0·597	0·934	Creatin-free food previous day
„ 30...	6,423	1039	Acid	122·8	27·3	1·284	0·661	0·623	„ „
July 4...	3,922	1039	Acid	121·3	19·6	0·901	0·431	0·470	„ „
„ 12...	2,842	1032	Acid	67·6	21·5	1·705	0·824	0·881	After diabetic diet for 4 days Weight, 7 st. 11 lbs.

1. The presence of sugar in the urine, unless in concentrations of above 5 per cent., does not materially affect the estimation of creatinin and creatin, provided always that the conditions laid down by Folin are strictly adhered to.

NOTES ON CASE I

This patient had been the subject of diabetes for six years. Before its onset she was a big, well-nourished woman weighing 15 st. (her own statement).

Polyuria was often excessive, and the output of sugar large. Acetone frequently occurred. There was a well-marked double cataract. The urine was first examined soon after her admission to hospital, while she was still on an ordinary mixed diet. The sugar and total nitrogen eliminated were both high. The total creatinin, on the other hand, rather low. The influence of withholding carbohydrate is seen at the second and third examinations, the total creatinin values are distinctly higher, the creatin readings showing a marked increase.

On creatin-free food a gradual fall in the excretion of creatinin and creatin may be observed, but the glycosuria increased and the diet was not persisted in.

The last examination was made after a return to diabetic diet; the total creatinin values were again increased. The weight was fully maintained during the period of observation.

TABLE II.—Female aet. 16. Weight, 7 st. 6½ lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			gram.	gram.	gram.	gram.	gram.	
May 22...	960	1034	Acid	28·5	7·4	0·562	0·531	0·031	Diabetic diet
,, 25...	1,620	1033	Acid	44·9	14·7	0·465	0·453	0·012	Creatin-free diet
,, 27...	1,136	1033	Acid	39·8	12·2	0·370	0·329	0·041	,, ,,
,, 31...	1,648	1035	Acid	41·2	15·9	0·388	0·316	0·072	,, ,,
June 18...	995	1029	Acid	14·2	8·4	0·576	0·548	0·028	Diabetic diet Weight 7 st. 6¼ lb.

NOTES ON CASE II

The first examination was made after the patient had been in hospital for three weeks. The glycosuria still persisted, but the other symptoms were in abeyance. The history dated back some ten months, but at no time had the illness been acute, and she was still fairly well nourished.

On ordinary diabetic diet the first analysis showed a low output of total nitrogen and creatinin. On creatin-free food the amount of total nitrogen eliminated suddenly shot up, and this was associated with a rise in the output of sugar and creatin. Creatinin itself is rather diminished. The sudden rise in the total nitrogen and creatin excretions during the period creatin-free food was being given may be explained by the fact that the diet was badly borne, and weight rapidly lost (5½ lbs. in five days). The last examination was made after the loss in weight had been made good, and the patient again on strict dietetic treatment. The total nitrogen output was again low, as was also that of creatinin.

TABLE III.—Male aet. 39. Weight, 13 st. 9 lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			gram.	gram.	gram.	gram.	gram.	
May 13...	3,000	1028	Acid	47·8	32·4	2·88	1·68	1·2	Diabetic diet
,, 19...	2,500	1031	Acid	35·4	37·5	3·74	2·22	1·52	,, ,,
,, 30...	2,600	1027	Acid	36·3	42·6	3·85	2·22	1·63	,, ,,
Nov. 24...	13,000	1030	Acid	335·6	56·6	3·21	1·98	1·23	Ordinary mixed diet
,, 26...	15,690	1033	Acid	506·7	47·07	2·28	1·47	0·81	,, ,,
,, 30...	10,000	1038	Acid	240·0	39·2	3·78	1·60	2·18	,, ,,
Dec. 3...	11,080	1035	Acid	250·0	65·3	4·66	2·59	2·07	,, ,,
,, 10...	10,520	1037	Acid	311·5	37·8	2·89	1·77	1·12	,, ,, Weight 13 st. 8 lbs.

NOTES ON CASE III

This was a case of acromegaly diabetes. Acromegaly and diabetes are so frequently associated that some close connection between the two diseases must exist. Von Noorden mentions five cases of acromegaly, four of which were complicated with diabetes. Various explanations to account for this relationship have been put forward. Some maintain that two entirely separate pathological conditions co-exist; others believe the glycosuria occurring in acromegaly to be produced by the pressure of the pituitary tumour on the fourth ventricle. A third view is that it is due to loss or increase of an internal ferment. Mayo Robson and Cammidge (16) state that chronic pancreatitis has been repeatedly observed in association with acromegaly, and are of opinion that the glycosuria in this disease is due to morbid changes in the pancreas.

When the patient first came under observation there was no glycosuria, but it soon appeared with all the classical symptoms of diabetes. The first three examinations were made when diabetic treatment was being tried, and the values of total nitrogen, creatinin, and creatin were all high. The patient then developed diphtheria, and it was four months later that the subsequent examinations were made. The enormous polyuria was the most striking symptom on his return, together with an appetite and thirst that can only be described as insatiable. On account of the latter no diet could be rigidly adhered to. He passed great quantities of sugar, the maximum being reached at the fifth examination. The total nitrogen eliminated reached great heights, the figure at the eighth examination being particularly noticeable; at the same time the total creatinin (creatin + creatinin) touched the highest level.

Creatin was always found in considerable amount.

It is worth observing that in spite of the man's symptoms the weight did not alter materially, and apart from the excessive craving for food he was quite comfortable.

TABLE IV.—Male aet. 30. Weight, 9 st. 12 lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			gram.	gram.	gram.	gram.	gram.	
May 13...	1,050	1020	Acid	Nil	18·1	1·843	1·57	0·273	Rich meat diet carbohydrate free
„ 19...	920	1022	„	Nil	16·7	1·267	1·16	0·107	„ „
„ 30...	1,365	1018	„	Nil	21·8	0·84	0·84	0·00	Eggs, milk, less meat
June 3...	1,194	1020	„	Nil	21·4	0·79	0·79	0·00	„ „
„ 5...	910	1025	„	5·0	15·7	0·618	0·58	0·038	Creatin-free diet
„ 6...	1,120	1027	„	8·3	16·3	0·613	0·57	0·043	„ „
„ 10...	870	1018	„	Nil	16·9	0·948	0·948	0·00	Mixed diet, with limited carbohydrate Weight, 9st. 12½ lbs.

NOTES ON CASE IV

This was apparently a case of so-called alimentary glycosuria, one in which a fair tolerance for carbohydrates existed. Lusk (v.s.) has pointed out that this condition must not be confounded with true diabetes. It occurs when the sugar holding capacity of certain organs is reduced or over-strained as when an excessive quantity of sugar is ingested.

When the patient first came under observation, sugar had been absent from the urine for eight days; this disappearance took place after twelve days' treatment. The diet consisted of protein

and fat, no carbohydrate being given. The presence of creatin at this time may have been entirely due to the excess of meat ingested (v.s.), but it is probable that the want of carbohydrate in the diet was also a determining factor. When eggs were substituted for some of the meat and milk added, the creatin disappeared, and at the same time the total creatinin diminished.

A creatin-free diet was now given and sugar again appeared in the urine. Creatin also reappeared, but the total creatinin excretion was somewhat reduced.

It is difficult to explain the reappearance of creatin on this carbohydrate rich diet, especially when the experimental work on dogs, referred to later, is taken into account. But in this case there is probably some definite pathological state underlying the condition, a state which only permits of the combustion of a certain (limited) amount of carbohydrate. If this limit be overstepped hyperglycaemia and glycosuria occur, and it may be that at the same time some catabolic process (possibly toxic) is originated which leads to a breaking-down of muscle protein and the appearance of creatin in the urine. The last examination made in this case was during a period of ordinary diet with limited carbohydrate. Both sugar and creatin were absent, and the total creatinin value was lower than that found when no carbohydrate was allowed.

TABLE V.—Female aet. 3. Weight 27½ lbs.

Date	Sp. gr.	React.	Sugar grams. per 1000	Total Nitrogen. per cent.	Total Creatinin. per cent.	Creatinin. per cent.	Creatin. per cent.	Remarks
October 12 ...	1028	Acid	20·0	0·42	0·023	0·015	0·008	Ordinary mixed diet
,, 13 ...	1028	,,	20·8	0·33	0·022	0·014	0·008	,, ,,
,, 19 ...	1029	,,	21·7	0·40	0·056	0·017	0·039	Creatin-free diet
,, 20 ...	1032	,,	25·0	0·53	0·022	0·010	0·012	,, ,,
,, 21 ...	1033	,,	29·4	0·58	0·026	0·008	0·018	,, ,,
,, 22 ...	1035	,,	33·2	0·73	0·013	0·005	0·008	,, ,,
,, 23 ...	1033	,,	30·0	0·79	0·016	0·007	0·009	,, ,,
,, 26 ...	1030	,,	28·7	0·99	0·023	0·015	0·008	After two days on ordinary diet. Weight 28 lbs.

NOTES ON CASE V

The first point of interest here is the age of the patient. On account of this the output of urine for the twenty-four hours could not be ascertained, and the table represents percentage values except for the sugar which is expressed in grams per 1,000 c.c. Diabetes at this age is rare. Amongst 1,360 private patients who consulted Pavy the disease began under ten years of age in only eight cases. The youngest patient Pavy had under his care was an infant aged 12 months. (17.)

The history in this case was of 8 weeks duration only, the chief symptom noted by the mother being excessive thirst.

It was easy to keep this patient on a creatin-free diet, but the glycosuria tended to increase. The total creatinin output, however, diminished.

The course of the illness was rapid, death ensuing from coma, at the end of six weeks after she first came under observation.

TABLE VI.—Male aet. 32. Weight 7 stone 13 lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			gram.	gram.	gram.	gram.	gram.	
Oct. 15 ...	2,103	1025	Acid	55·1	10·6	0·455	0·428	0·027	Diabetic diet
,, 27 ...	2,160	1028	,,	50·5	15·4	0·619	0·591	0·028	,, ,,
,, 30 ...	2,100	1028	,,	44·6	13·8	0·612	0·584	0·028	,, ,,
,, 31 ...	3,520	1030	,,	102·0	18·3	0·564	0·529	0·035	Creatin-free diet
Nov. 1 ...	3,200	1032	,,	112·0	10·9	0·331	0·280	0·051	,, ,,
,, 2 ...	3,240	1032	,,	111·7	10·4	0·298	0·247	0·051	,, ,,
,, 3 ...	2,100	1028	,,	41·4	9·7	0·425	0·402	0·023	Diabetic diet, modified Weight 8st. 0 lbs.

NOTES ON CASE VI

This patient had been in hospital for five weeks before he came under observation. He was doing well on a strict diabetic diet, but the glycosuria was still marked. Although the intake of flesh was considerable, the readings are not high; the total creatinin excretion being low, and the nitrogen elimination well within normal limits. On a creatin-free diet the amount of urine and sugar passed both increased; the total creatinin output on the other hand was lower. On the day following the application of creatin-free food there was a rise in the output of total nitrogen; this rise, was however, transitory.

The final examination shows the effect of a diabetic diet with the addition of small quantities of carbohydrate. It is again evident from this table that a diabetic even on a rich nitrogenous diet need not excrete excessive amounts of nitrogen and creatinin.

TABLE VII.—Male aet. 33. Weight, 8 st. 6¼ lbs.

Date	Amount urine	Sp. gr.	React.	Sugar	Total nitrogen	Total creatinin	Creatinin	Creatin	Remarks
	c.c.			gram.	gram.	gram.	gram.	gram.	
Nov. 9 ...	4,830	1039	Acid	182·4	14·8	0·710	0·648	0·062	Ordinary diet; acetonuria
,, 11 ...	3,500	1042	,,	115·5	15·3	1·070	0·850	0·220	do. do.
,, 12 ...	2,700	1040	,,	91·8	10·05	0·820	0·653	0·167	Ordinary diet; no acetone
,, 16 ...	800	1055	,,	45·0	7·9	0·759	0·338	0·421	Diabetic diet; no acetone
,, 17 ...	1,140	1040	,,	23·1	14·6	1·084	0·534	0·550	Diabetic diet; acetonuria
,, 19 ...	800	1040	,,	17·6	6·9	0·608	0·242	0·366	Diabetic diet; marked acidosis
,, 23 ...	760	1039	,,	16·1	6·2	0·787	0·242	0·545	Carbohydrate and alkali; marked acidosis
,, 24 ...	1,650	1042	,,	82·5	11·7	1·025	0·557	0·468	Carbohydrate and alkali; acetonuria
,, 26 ...	3,500	1045	,,	157·5	22·0	1·869	0·942	0·927	Ordinary diet; no acetone Weight, 8 st. 0½ lb.

NOTES ON CASE VII

For some days this patient was seriously ill, and unfortunately left hospital (of his own accord) before his condition was considered safe, and before a creatin-free diet could be tried.

The amount of urine passed showed considerable variations as did also the output of sugar and total nitrogen, the latter being generally low.

The acetonuria present at the outset disappeared, and a carbohydrate-free diet was given. This reduced the glycosuria but led to an increase in the output of creatin, and a marked acidosis, the breath having the typical smell. Carbohydrates and alkalis were pushed, and the acidosis gradually disappeared. The various readings in this case were somewhat irregular, probably on account of the extreme perversion of metabolism present.

TABLE VIII.—Male aet. 37. Weight, 7 stone 9 lbs.

Date	Amount urine c.c.	Sp. gr.	React.	Sugar gram.	Total nitrogen gram.	Total creatinin gram.	Creatinin gram.	Creatin gram.	Remarks
Nov. 16 ...	3,400	1038	Acid	170·0	11·9	0·669	0·350	0 319	Ordinary diet; trace acetone
,, 17 ...	3,400	1035	,,	163·2	17·0	0·800	0·498	0·302	Ordinary diet; no acetone
,, 19 ...	3,444	1036	,,	61·0	17·1	0·986	0·478	0·508	Diabetic diet; no acetone
,, 23 ...	3,410	1037	,,	92·0	15·6	1·031	0·470	0·561	,, ,,
,, 24 ...	2,850	1036	,,	81·2	18·8	1·184	0·652	0·532	,, ,,
,, 26 ...	3,000	1040	,,	78·1	17·9	1·447	0·698	0·749	Diabetic diet, acetonuria
,, 30 ...	3,350	1039	,,	75·9	17·7	1·517	1·609	0·908	,, ,,
Dec. 3 ...	3,750	1038	,,	92·5	27·0	1·728	0·738	0·990	Carbohydrate and alkali; acidosis
,, 10 ...	3,700	1040	,,	85·1	25·2	1·913	0·773	1·14	,, ,,
,, 15 ...	3,100	1037	,,	71·3	19·9	1·573	0·550	1·023	,, ,, (less)
,, 16 ...	3,500	1038	,,	157·5	14·7	0·741	0·413	0·328	Ordinary diet; no acetone Weight 7 st. 6 lbs.

NOTES ON CASE VIII

Like the preceding case this patient was acutely ill for some days, and in the same way left hospital before a creatin-free diet could be given.

He had suffered from diabetes for four years, with well-marked symptoms, and at the time of examination was considerably reduced in weight.

On ordinary diet he was progressing favourably, though a large amount of sugar was still being passed. When the urine became free of acetone a diabetic diet was tried. The immediate result of this was to reduce the glycosuria, but a marked and progressive rise in the excretion of creatin took place, together with all the signs of acid intoxication. This high elimination of creatin was most marked when the acidosis was at its height. The effect of a nitrogenous (meat) diet on the total creatinin excretion is well shown.

TABLE IX.—Male aet. 50. Weight, 9 st. 4 lbs.

Date	Sp. gr.	React.	Sugar grams per 1,000 c.c.	Total Nitrogen per cent.	Total Creatinin per cent.	Creatinin per cent.	Creatin per cent.	Remarks
December 16 ...	1038	Acid	28·0	0·30	0·0144	0·012	0·0024	Modified diabetic diet
,, 18 ...	1045	,,	23·4	0·40	0·0289	0·025	0·0039	,, ,,
,, 20 ...	1042	,,	37·02	0·36	0·0167	0·013	0·0037	Creatin-free diet
,, 21 ...	1047	,,	32·06	0·58	0·0328	0·029	0·0038	,, ,,
,, 23 ...	1043	,,	35·7	0·40	0·0249	0·021	0·0039	,, ,,
,, 24 ...	1040	,,	36·3	0·36	0·0212	0·016	0·0052	,, ,,
,, 27 ...	1042	,,	31·2	0·33	0·0197	0·014	0·0057	,, ,,
,, 29 ...	1040	,,	32·2	0·40	0·0265	0·019	0·0075	,, ,, Weight, 9 st. 4 lbs.

NOTES ON CASE IX

This patient was the only case in the present series who was not an hospital in-patient. He was a tailor to trade, and attended at the out-patient department. When first seen he was on a modified diabetic diet, carbohydrates being restricted as far as possible, and under this treatment he was doing well. An effort was made to measure the quantity of urine passed in the twenty-four hours, but the results were unsatisfactory, so that only percentage amounts are represented in the table. It was apparent, however, that there was considerable polyuria. His illness dated back five years, but had never been severe, and he was always able to follow his employment. The figures obtained are fairly uniform. Creatin was always found, and tended to increase slightly with creatin-free diet, this increase being associated with a rise in the output of sugar.

TABLE X.—Male, aet. 22. Weight, 8 stone 6 lbs.

Date	Amount urine c.c.	Sp. gr.	React.	Sugar gram.	Total nitrogen gram.	Total creatinin gram.	Creatinin gram.	Creatin gram.	Remarks
Dec. 4 ...	2,900	1,039	Acid	80·0	17·1	1·155	0·715	0·440	Diabetic diet
,, 5 ...	2,950	1,038	,,	81·2	17·1	1·173	0·716	0·457	,, ,,
,, 6 ...	2,950	1,034	,,	82·6	23·6	0·982	0·581	0·401	,, ,,
,, 7 ...	2,950	1,035	,,	97·3	20·0	0·805	0·486	0·319	Creatin-free diet
,, 8 ...	2,850	1,036	,,	88·3	19·9	0·943	0·561	0·382	,, ,,
,, 9 ...	2,850	1,035	,,	91·2	19·9	0·741	0·451	0·290	,, ,,
,, 10 ...	2,800	1,040	,,	100.8	18·4	0·696	0·414	0·282	,, ,,
,, 11 ...	3,200	1,036	,,	102·4	19·9	0·708	0·464	0·244	,, ,,
,, 12 ...	3,000	1,035	,,	84·0	21·4	1·020	0·504	0·516	Diabetic diet
,, 13 ...	2,800	1,038	,,	81·2	20·6	1·004	0·506	0·498	,, ,, Weight 8 st. 7 lbs.

NOTES ON CASE X

A history extending over two years was given by this patient. When first seen he was on diabetic diet, but the polyuria and glycosuria were still abundant. The total creatinin output on this diet cannot be considered large, nor was the elimination of nitrogen excessive. On creatin-free food the excretion of total creatinin fell, both creatinin and creatin being diminished in amount. With a return to diabetic diet these values both became higher.

TABLE XI.—Male aet. 23. Weight, 7 st. 8½ lbs.

Date	Amt. Urine c.c.	Sp. gr.	React.	Sugar grams.	Total Nitrogen grams.	Total Creatinin grams.	Creatinin grams.	Creatin grams.	Remarks
Jan. 28 ...	7,000	1038	Acid	140	27·3	1·484	0·714	0·770	Ordinary diet, carbohydrate limited
„ 29 ...	6,000	1035	„	150	24·0	1·512	0·672	0·840	„ „
„ 30 ...	6,000	1036	„	204	18·0	1·260	0·588	0·672	Creatin-free diet
„ 31 ...	5,500	1037	„	191·4	19·2	1·072	0·566	0·506	„ „
Feb. 1 ...	6,500	1036	„	208	19·8	1·084	0·576	0·508	„ „ Weight 7 st. 7½ lbs.

NOTES ON CASE XI

Although this man had only been in hospital for a week, he refused further treatment, and a more extended examination was impossible.

Like the previous case, he was a poorly nourished man, with a history of about two years' duration. He passed a large amount of urine, and the glycosuria was also great.

On ordinary diet the total nitrogen excretion was large, but the total creatinin output was not high. The large proportion of creatin is noticeable. On creatin-free diet the output of total nitrogen diminished, and the creatinin and creatin values were also reduced.

The patient was losing weight, and the prognosis was considered unfavourable.

The above tables represent the majority of the cases examined for this investigation. It will at once be seen that creatin was always present—sometimes to a considerable extent—when sugar occurred in the urine. The fact that creatin was found even after a patient had been on a creatin-free diet for some days is sufficient indication that it must be under these conditions derived from the tissues and not from the food ingested.

The effect of a creatin-free diet was generally to decrease the output of both creatinin and creatin, but the latter was sometimes increased. This is probably due to the fact that a suitable creatin-free diet is (as has already been noted) of necessity rather rich in carbohydrate, and tends to increase the glycosuria, and in some instances upset the patient.

With the exception of the case of acromegaly diabetes, the total creatinin eliminated by these patients cannot be considered high; even on strict diabetic diet these cases did not eliminate an excessive amount of creatinin.

The case of acromegaly diabetes must be taken separately; the figures in this case reached great heights; but it is reasonable to suppose that the diseased pituitary gland contributed to this great increase in

metabolism, either of itself or in association with a morbid condition of the pancreas.

As a result of this investigation the following conclusions may be drawn.

1. Creatin is constantly found in the urine of diabetics.

2. The presence of creatin in diabetic urine on a creatin-free diet is an expression of increased endogenous protein catabolism due to inability on the part of the tissues to burn sugar. Or it may be due to the non-conversion of creatin into something else, just as β-oxybutyric acid is improperly converted in certain abnormal states of metabolism.

3. Total creatinin excretion is not abnormally increased in diabetes even when a diet rich in flesh diet is being ingested.

4. The amount of creatinin excreted, even on a creatin-creatinin-free diet, varies to a certain extent with the nitrogen intake.

NOTE.—Since this paper was sent in for publication there has appeared in the *Quarterly Journal of Experimental Physiology* a paper by Krause on the presence of creatine in the urine of patients suffering from diabetes mellitus.

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ON THE QUANTITATIVE ESTIMATION OF UROBILIN IN THE EXCRETA AND ITS VALUE AS A MEASURE OF HAEMOGLOBIN METABOLISM

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I.—Introduction

Many acute and chronic diseases are associated with a fall in the haemoglobin of the blood as determined by the haemoglobinometer, but the exact value of these figures is rendered unreliable, especially in acute febrile diseases, by alterations in the blood volume; in addition the haemoglobinometer determination cannot give information about the fate of the haemoglobin which may be destroyed or possibly used again for new corpuscles.

For further knowledge of this question it is necessary to consider the remote excretory products of haemoglobin; of these the derivatives of the protein portion would be merged to an unrecognisable extent in the general protein metabolites while the iron of the pigment is shown by post-mortem findings to be largely retained, especially by the liver and spleen,[15] and the portion eliminated is of small value as a measure of the total destruction.

The remaining constituent of the haemoglobin is the iron-free pigment moiety, and, though a portion of this is probably retained in the body, much of it finds its way into the excretae in various forms under normal conditions, especially when active haemolysis is proceeding; it is excreted from the liver as bile pigment, but during its passage through the intestinal canal it is subjected to the action of micro-organisms, and except in conditions of increased peristalsis and in the new-born (before the intestinal fauna has developed) but little is evacuated in this form; the majority being changed to urobilin or its chromogen, and being eliminated in this form by the faeces or after reabsorption by the urine.

Urinary urobilin has attracted much attention since its discovery by Jaffe[1] in 1868, and the question of its relationship to faecal urobilin gave

rise to some controversy, but it has now been shown that urobilin of identical composition can be separated from urine and faeces and also in small amounts from bile; under various pathological conditions—pyrexia, internal haemorrhage, cirrhosis, and other hepatic disorders, anaemias and constipation—and also after ingestion of haemoglobin (blood sausage)—the urobilin of the urine tends to be increased in quantity; diarrhoea, obstruction of the bile passages, and some conditions of grave hepatic disorder usually lead to a diminution, while in complete stenosis of the bile ducts and in the new-born it is entirely absent from both urine and faeces.

Hildebrandt[2] gives an exhaustive summary of previous work and an excellent statement of the present state of our knowledge in his article on 'Urobilin and Icterus,' and has greatly advanced the appreciation of its clinical significance by his extensive study of the urines in most diseases of temperate climates; unfortunately, however, he gives no quantitative figures, and did not follow the faecal excretion in his cases.

Hoppe Seyler,[3] Muller and Gerhardt[4] and others have estimated quantitatively the urinary excretion of urobilin in health and disease, and found amounts varying from 10 to 130 mgm. per diem in the urine of healthy adults; in ordinary pyrexial diseases (e.g. pneumonia), in anaemias, hepatic cirrhosis, and internal haemorrhage from 300 to 500 mgm. per diem were frequently observed, while the highest figures given are 800 mgm. per diem in scarlet fever, and 2,000 mgm. per diem in perityphlytis. In malaria, however, De Jonge[5] appears to have found amounts as high as 6,000 mgm. per diem when quinine was being administered.

The amount of quantitative work on faecal urobilin has by no means equalled that on the urinary; Hildebrandt scarcely refers to it at all, and the only figures that have been found in the literature are the following: 21 to 29 mgm. per diem in chlorosis, and 153 to 900 mgm. in pernicious anaemia, contrasted with 30 to 124 mgm. and 153 mgm. respectively in the urines (French[6]).

II.—Methods of Investigation

(*a*) *Urobilin in the Urine.*—Urobilin may be precipitated from the urine by zinc-chloride,[1] or mercuric salts, or by saturation with ammonium sulphate and the precipitate then further purified and weighed (Mehú[7]): alternatively the precipitate obtained by the above methods may be dissolved in alcohol or other solvent, and estimated by

the spectrophotometer, as was done by Hoppe Seyler,[3] by Gerhardt and Muller.[4]

Other observers have used the fluorescence obtained with zinc salts as a quantitative test, by determining the maximum dilution of the urine at which such fluorescence is obtainable, and comparing this with a standard solution of purified urobilin under similar conditions.

The gravimetric and spectrophotometric processes are rather too long for clinical work, and in my hands the fluorescence tests proved rather variable, and so were only used occasionally as a more delicate check on the results obtained by the following method.

Ten cubic centimetres of a twenty-four hours' specimen of urine were acidified with sulphuric acid, and allowed to stand for some time fully exposed to daylight to convert the chromogen to urobilin; the urine in a layer 15 millimetres thick was then examined by a Zeiss direct vision spectroscope in a good light, and if a urobilin band was present it was diluted gradually with water until the dilution was found at which the band was just perceptible. The spectroscope was standardised by a solution of purified urobilin in acidulated water, separated by the method described by Garrod and Hopkins[8] from the urines of two cases with marked urobilinuria.

The values given by this method appear to be fairly accurate when the concentration of urobilin is such that a dilution of fifteen to twenty volumes or more is necessary to extinguish the band, but at lower concentrations they are considerably below the correct figure, owing to the band being masked by the absorption due to other pigments; and urine of normal adults examined by this method appears to contain no urobilin.

With the spectroscope used in these experiments, the band of acid urobilin was just perceptible when a layer 15 mm. in depth of a solution of 10 mgm. of purified urobilin per litre was examined. Viglezio[9] and Gerhardt and Muller[4] give the delicacy of the spectrophotometer as 5 mgm. per litre; Saillet[10] gives 45 mgm. per litre for the spectroscope; Schlesinger[11] 20 mgm. per litre for fluorescence with zinc acetate, and other observers give 10 mgm. per litre as the limit for fluorescence with zinc chloride and ammonia.

Jaffe's test (fluorescence with zinc chloride and ammonia) was found in my hands to have a delicacy varying from 10 to 3 mgm. per litre; and Schlesinger's test (fluorescence with zinc acetate) was very reliable, with a delicacy of about 2·5 mgm. per litre; but, unfortunately, it was not tried till late in the series, and was only used as a check in later cases.

A very useful qualitative test, which might also be used quantitatively, is the change of colour obtained on the addition of a mercuric salt (Millon's reagent) to dilute solutions of acid urobilin. The change from the brown colour of acid urobilin to the definite pink of mercury urobilin is very striking, especially if a large vessel is used.

(*b*) *Urobilin in the Faeces.*—As has been already stated, the literature dealing with the excretion of urobilin by the faeces is very much less extensive than that of urinary urobilin, probably owing to the greater difficulties of the examinations.

Urobilin may be extracted from the faeces by many solvents, and those which have been mostly used are alcohol, amyl-alcohol, alcoholic ammonia, and ammonia. McMunn[12] deals exhaustively with the various spectroscopic appearances of these extracts in his important contributions to the literature of urobilin and in his papers alone has any mention been found of extracts of faeces which had apparently a urobilin content comparable in amount to those which have been met with in these experiments: the extracts he obtained with acid alcohol especially showed a very intense absorption.

Ammonia and the organic solvents, however, have no selective action and many other pigments present in the faeces are also taken up by them and obscure the results obtained—bile pigment, chlorophyll, and haematoporphyrin especially are frequently found.

After trials of various solvents it was found that extracts made with dilute acid gave the most satisfactory result: this was chosen because neither bilirubin nor chlorophyll are dissolved by it to any extent. The day's faeces were well shaken with water, and dilute sulphuric acid added until effervescence had ceased and the reaction was distinctly acid, and the vessels were allowed to stand for one or two days exposed as fully as possible to daylight to convert the chromogens to urobilin: (a very definite change of colour from pale yellow or green alkaline stools to a red-brown was often observed). The mixture was then filtered into glass vessels fully exposed to light, and the residue shaken repeatedly with further amounts of dilute acid until the filtrates were but very slightly coloured, even after exposure to light (if the same filter paper be used throughout a fairly reliable idea of the end point is obtainable by noting when the filter paper on the outside is nearly white). The mixed extracts (or in some cases each extract separately) of the day's faeces were tested by the spectroscope in the manner described for estimation of urinary urobilin. The amount of solvent used varied as a rule between

one and six litres, and in the faeces of febrile cases especially the concentration of urobilin was very high. The colour varied from a light brown to a deep brown, occasionally a reddish-brown or port wine coloured extract was obtained; in some cases the extract deposited a red-brown sediment on standing—probably bile pigment, owing to the use of insufficient acid. The insoluble residues were dark in colour, often of a grey muddy appearance and red flocculi (bilirubin?) could often be distinguished. Subsequent extraction with ammonia was tried in some instances, the residue then remaining was very nearly colourless on some occasions, and once or twice was very small in amount; the ammoniacal extract was rather viscous, of a dark green or olive colour, and when examined with the spectroscope showed a rather widespread general absorption and no definite bands; on acidification a brown precipitate usually settled, and the solutions usually then showed some urobilin band, but no very careful investigations have been made, bile pigment was certainly present in some cases. It is possible that in the prolonged process of extraction with dilute acid some bile pigment may be changed into urobilin, but such change, if it occurs, would, for the purposes of this research, have been distinctly advantageous.

When examined by the spectroscope at a sufficient degree of dilution the usual band at F of acid urobilin was present, though in one or two cases it was not so sharply defined as is usually found; occasionally the left hand (red) border was a little nearer 'b' than usual, and in one case especially this was very marked, and caused considerable trouble until its association with a very red coloured extract was noticed; the band was found to coincide with that of mercury urobilin, and on enquiry the administration of calomel was confirmed. Probably the slight variations of the left-hand border of the band are associated with the presence of urobilin metallic compounds.

A high dilution is usually necessary before the urobilin band becomes defined, in the original extracts the blue end of the spectrum was only on rare occasions perceptible, and in the majority of instances only a portion of the red part of the spectrum was visible; as dilution proceeded the general absorption got less and less, and the urobilin band became defined. In only one instance (out of some eighty stools examined) was any other band than that of urobilin detected in the extracts; in the three last washings of the exceptional stool of the case of Blackwater Fever, which will be mentioned again later, a band was visible immediately to the right of Fraunhofer's D line up to a dilution of five volumes. When

undiluted the band crossed the D line and extended a little to the left. In an extract of a portion of the residue of the faeces of this case with ammoniacal alcohol, three bands were found corresponding very closely with those of alkaline haematoporphyrin.

In its spectroscopic appearances the urobilin of the faeces obtained by this process of extraction appears to be identical with that of the urine, but in some other respects this resemblance does not obtain: the fluorescence of faecal urobilin with zinc salts (especially with zinc acetate) is more marked than is that of urinary, and it does not behave to different solvents like urinary urobilin.

Garrod and Hopkins[8] have elaborated a method of obtaining urobilin in a pure form from various secretions containing it, and their quantitative analyses of such pure products gave identical results. They found, however, more especially in the faeces, various modifications of urobilin differing slightly from normal urobilin in solubilities and in spectroscopical appearances. By various manipulations (e.g., evaporation of a chloroform solution), some of these modifications have been found to be converted to normal urobilin. In the extracts obtained from the faeces of malarial patients the urobilin appears to exist in large part in such modified forms, and as yet we have failed to obtain sufficient pure urobilin from them for quantitative analysis; the difficulty occurs in the extraction of urobilin from acid solutions saturated with sulphate of ammonia by chloroform ether, as described by Garrod and Hopkins; the bulk of the precipitate could not be induced to enter into solution in the organic solvent; usually this obtained at the first attempt, but on two occasions the urobilin passed rapidly and fully to the organic solvent, and was extracted from that by distilled water, only, however, on the addition of a rather large amount of sodium bicarbonate. Saturation with ammonium sulphate and acidification caused the usual precipitate easily transferred to ether-chloroform: the urobilin was transferred with difficulty from this to ammoniacal distilled water, only on the addition of a considerable amount of ammonia: on acidification of the ammoniacal solution a red flocculent precipitate settled, and only a small amount of urobilin could be obtained in chloroform solution: the red precipitate could be dissolved in alkaline (but not to any extent in acid solutions, even strongly acid), and again reprecipitated by acidification; all the clear solutions, chloroform, alkaline, and acid, showed their usual urobilin bands, the turbid solution showed clearly a band exactly

corresponding to the urobilin E band of Garrod and Hopkins, *as figured* in Plate I, fig. B, of their contribution describing it.[8]

In other cases the urobilin proved refractory in large measure on its first precipitation, but in no other case could such additional bands be seen. We have not as yet succeeded in obtaining pure faecal urobilin (and in consequence our figures may need some slight revision owing to a possible variation in its absorptive power), and it certainly appears that though the faeces do contain some urobilin identical with urinary urobilin, they also may contain large amounts of other urobilins differing from urinary urobilin in their properties.

III.—Urobilin Output. Its Variations and their Significance.

By these methods the urine and faeces were investigated in a number of healthy adults and in a number of patients suffering from various diseases, and the following figures among others were obtained for the excretion of urobilin per diem:—

Nature of case	Urinary Urobilin in mgs.	Faecal Urobilin mgs.
Normal adults (8 cases)	None found	50 to 100
Pneumonia (Temp. 103° F.)	100 to 120	?
Pneumonia with delirium (Temp. 104° F.) ...	60 to 190	80 to 85
Pneumonia (Temp. 104° F.)	36 to 75	?
Malta fever (Temp. 104° F.)	100 to 150	310 to 350
*Tonsillitis (Temp. 103° F.)	160	—
Malignant endocarditis (Temp. 102° F.)	23	—
Anaemia (Gastro-intestinal origin)	—	130 to 40
Pernicious anaemia (2 cases)	5 to 11	—
Intramuscular haemorrhage (post operative) ...	nil	120
Morbus cordis, hepatic congestion	18	—
Acute suppurative hepatitis (Dysenteric) (T. 104° F.)	155	80
Yellow fever (old specimen; much bile)	42	—
Chronic malaria (2 cases quiescent)	none	50 to 150
*Chronic malaria (quiescent; very anaemic) ...	20	150

*These two patients had previously had blackwater fever.

These figures, as will be noticed, are quite comparable with those obtained by other observers for similar conditions; if anything, they are lower than previously recorded results: it will be further noted that the faecal output as a rule is considerably larger than the urinary.

Quantitative Relationship of Urobilin and Haemoglobin. Since urobilin is the excretory representative of a definite portion of haemoglobin it is possible to determine what proportion of the haemoglobin of the

body is represented by a known weight of urobilin, and so measure roughly the haemoglobin breakdown represented by any known excretion of urobilin.

Haemoglobin is present in the blood and muscles, and in a man of 60 kilograms, in health, there will be about 760 grams of haemoglobin, (blood = 1/12 body weight, and haemoglobin = 15 per cent. of blood-weight);[13] the quantity in the muscles is rather more difficult to estimate, but we know that muscle contains about 0·05 gram of iron per kilogram,[13] and in a 60 kilogram man this would represent not more than 380 grams of haemoglobin. In addition to these amounts we have the bile pigment and its precursors in the liver and bile passages, and as about one gram of bile pigment is excreted a day by a normal man,[14] probably it is fairly correct to assume that about five grams of urobilin is obtainable from this source; to this may be added the urobilin (4 per cent.) obtainable from the haemoglobin of the blood and of the muscles—30 grams and 15 grams respectively—and we get a possible output from a normal man of 50 grams of urobilin, of which 30 grams is represented by the haemoglobin of the blood.

In health the normal daily excretion of urobilin (100 to 200 mgm.) represents about 0·2 to 0·4 per cent. of the total haemoglobin, or of 0·3 to 0·7 per cent. of the haemoglobin of the circulating blood.

In diseases this amount is often more than doubled, rising in the case of Malta fever as high as 500 mgm., or 1·5 per cent. of the total blood; and probably further observations on faecal urobilin in disease will yield still higher figures.

IV.—Malaria

Twenty-eight cases of malaria, occurring in the Royal Southern Hospital, were investigated by this method in the hope that more light might be thrown on various problems connected with this disease: the urinary urobilin output alone was unfortunately investigated in the majority of the cases, and it was not till late in the series that the faecal channel was also considered, and only in five cases have continuous observations been made.

The full results are recorded, and their bearing on malaria considered, in other papers,[16] and only a few important points will be alluded to here.

There is in the period of and subsequent to malarial pyrexia, a rise in

the output of urobilin by the urine, though as a rule this does not attain to any very high figure.

In a few cases of malignant tertian malaria (*P. falciparum*) a very high output of urobilin by the urine was observed on one or two days (not necessarily associated with constipation); the high figures recorded in these cases were respectively 2300, 900, 2000 and 1730 mgm. per diem: at the time, De Jonge's[5] paper had not been seen, and these results appeared rather astonishing, though much smaller than those recorded by him. They differed from his, however, in that the outputs were uniformly higher in malignant tertian than in benign tertian infections (*P. vivax*), and that they occurred apart from the administration of quinine.

The faeces were investigated on a few occasions in seven of the cases, and the faecal output of urobilin was invariably found to be much higher than the urinary. In benign tertian malarias the increase was seldom more marked than in other diseases with a similar degree of pyrexia; in malignant tertian malaria the outputs were much higher and frequently reached more than 1000 mgm. per diem immediately after the pyrexial period. In many cases the figures immediately after pyrexia while on milk diet were found to be very much larger than the normal urobilin output (50-100 mgm.) of the same patients when convalescent and on full meat diet. Four cases, two of benign tertian malaria and two of malignant tertian, were investigated throughout the attacks (though some few stools were lost by unavoidable circumstances).

The output in the benign tertian cases is not marked, the highest day's yield being 530 mgm., and the total output in the week following the onset of the pyrexia being respectively 1220 mgm. and 1300 mgm., equivalent to 4·2 per cent. and 4 per cent. of the patient's total circulating haemoglobin.

In the malignant tertian cases the output is very high (and in each the urinary output on one day is marked), though in one fairly evenly distributed over the early stools and in the other mainly concentrated in one stool. The highest day's outputs by the faeces are in Case III, 1,730 mgm., and in Case IV 4,100 mgm., while the total outputs in the week after the onset of the pyrexia amount to 6,400 and 6,800 mgm., equivalent to 26 per cent. and 18 per cent. respectively of the total normal circulating haemoglobin, and corresponding very closely with the falls of 25 per cent. and 20 per cent. recorded by haemoglobinometer determinations. The daily outputs in the periods of convalescence and on full diet were respectively 20 and 150 mgm. of urobilin.

These figures are in themselves worthy of note, but further stress must be laid on the difference between the urinary and faecal outputs, the latter representing 55 per cent. of the total output in Case III, and 60 per cent. in Case IV, in spite of the fact that these were two of the four cases in which extremely high outputs of urobilin occurred by the urine.

The following particulars are given for the extract obtained from the stool of Case III (day 1). Two litres of mixed extract were obtained, in which—

The band of acid urobilin was visible to a dilution of 100 vols. (equals 200 litres).
The band of mercury urobilin was visible to a dilution of 120 vols. (equals 240 litres).
The pink colour of mercury urobilin was clearly visible to a dilution of 800 vols. (equals 1,600 litres.)
And fluorescence with zinc chloride and ammonia was clearly visible to a dilution of 300 vols. (equals 600 litres).

The fifth case alluded to is of particular interest, and has been reported by Major Ross, Dr. D. Thompson, and myself;[17] this patient, while under observation, had first an attack of ordinary malignant malaria, and then one of blackwater fever. Some of the stools were unfortunately lost, but from those obtained it may be seen that the amount of urobilin excreted in the first instance represented about 10 per cent. of the total blood, while that in the blackwater attack represented over 100 per cent. of the haemoglobin of his blood. The majority of the excretion in both instances was by the faeces, and in the second attack nearly all was abstracted from one stool already referred to as showing also the bands of haematoporphyrin: the bulk of extract obtained from this stool and its urobilin contents are so astonishing that they will be submitted in more detail: ten successive extracts of this stool were made in all, and they can be sub-divided into three divisions:—

4,000 c.c. showing the band to 400 dilutions, fluorescence with zinc acetate to 1,600 dilutions.
7,400 c.c. showing the band to 200 dilutions, fluorescence with zinc acetate to 1,000 dilutions.
3,000 c.c. showing the band to 40 dilutions, fluorescence with zinc acetate to 600 dilutions.

In all equivalent to 3,200 litres of fluid, showing the urobilin band in a layer 15in. thick, and 15,600 litres of fluid showing green fluorescence with zinc acetate when examined in layers of the same thickness.

This corresponds to 32 grams of urobilin, whereas his normal excretion on full meat diet was only 180 mgms. a day.

In his first attack the haemoglobin estimation fell from 85 to 70 per cent., in the second from 75 to 25 per cent., and the red corpuscles fell as low as 800,000 per c.mm.; while 100 per cent. of his haemoglobin was

represented by urobilin of the excretae and the patient was intensely jaundiced and vomiting some bile, the total haemoglobin passed unaltered in the urine represented only about 20 c.c. of blood or under 0·5 per cent. of his total circulatory haemoglobin.

This result gives very strong grounds for concluding that the blackwater fever in this case was merely an overflow owing to haemoglobin being set free faster than the liver could deal with it, and further seems to show that the insufficiency of the liver was due to no lack of function from disease.

Conclusions.

1. The urobilin of the urine is useless as an indication of the total haemoglobin metabolism. (It does serve, however, as an indication of the absorptive capacity of the intestinal wall (Hildebrandt).)

2. Occasionally, and not necessarily in association with constipation, a large amount of urobilin passes away in the urine, and so in quantitative work it is necessary to watch the urine for any such manifestation.

3. Save for such occasional large urinary excretions, most of the urobilin is excreted in the faeces, and the total output of urobilin would appear to represent very closely the total breakdown of haemoglobin.

4. The pigment moiety of haemoglobin set free in the body by haemolysis appears to be almost entirely excreted within a few days.

5. The liver can deal with a very large amount of haemoglobin before any overflow of unaltered haemoglobin occurs in the urine.

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THE PHYSIOLOGICAL ACTION OF SOME CAMPHENE PHOSPHONATES OF SODIUM

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I.—INTRODUCTION.
II.—EFFECT OF INTRAVENOUS INJECTIONS.
III.—EFFECT ON THE HEART.
IV.—EFFECT OF SUBCUTANEOUS INJECTIONS.
V.—SUMMARY AND CONCLUSIONS.

I. Introduction

In a paper published in 1894,[1] Marsh and Gardner showed that when camphene is treated in the cold with phosphoric pentachloride, one atom of hydrogen of the camphene is replaced by the grouping PCl_4 with the formation of a compound of the formula $C_{10}H_{15}PCl_4$, which on treatment with water yielded two acids—*a*- and *β*-camphene-phosphonic acids. The *a*-camphene phosphonic acid crystallised from dilute alcohol in colourless fluffy needles, insoluble in ether, but soluble in alcohol, benzene and chloroform. The air-dried acid had the composition represented by the formula $2C_{10}H_{17}PO_3H_2 + H_2O$. When dried at 100° it lost $2H_2O$, leaving the anhydro-acid $(C_{10}H_{15}PO_2H)_2O$. The acid was found to be monobasic, and yielded a sodium salt of the formula $C_{10}H_{15}PO_3HNa + 4H_2O$. When heated at 100° the salt loses its water of crystallisation, two molecules of the salt losing $9H_2O$ and leaving the salt of the anhydro-acid $(C_{10}H_{15}PO_2Na)_2O$.

β-camphene-phosphonic acid has the formula $C_{10}H_{15}PO_3H_2$, and crystallises from dilute alcohol in large colourless needles. It is soluble in ether, but insoluble in chloroform and benzene. It melts with decomposition at 167°, and does not yield an anhydro-acid. Like the *a*-acid, it is monobasic and yields a sodium salt which crystallises from water in unctuous plates having the formula $C_{10}H_{15}PO_3NaH + 5H_2O$. At 100° this salt loses nearly the whole of its water of crystallisation, but the last traces are difficult to remove at a temperature below that at which the salt itself undergoes decomposition.

1. *Trans. Chem. Soc.*, London, p. 35, 1904.

The *a*- and *β*-acids have not been converted the one into the other.

The *β*-sodium camphene-phosphonate decomposes quantitatively at 160° C. into camphene and sodium metaphosphate. The *a*-salt is more stable; on heating it yields the salt of the anhydro-acid, which does not decompose below 260° C., and then the decomposition is of a more profound nature.

On hydrolysis with water in sealed tubes both salts decompose with the formation of camphene and sodium hydrogen phosphate. Both salts are decomposed by bromine water with the liberation of phosphoric acid and the formation of bromo derivatives of camphene. The phosphonic acids derived from different camphenes differ in their rotatory power in respect of polarised light, but do not differ materially in chemical properties.

The above-mentioned properties may be explained on either of two views: (1) The replacement of OH of orthophosphoric acid by a camphene residue gives rise to two isomeric acids of the formula $O=P\langle^{C_{10}H_{15}}_{(OH)_2}$ of which the *a*-acid readily loses water on heating, passing into the pyro-form, while the *β*- does not do this at any temperature below which it decomposes; or (2) the *a*-acid may be regarded as derived from pyrophosphoric acid by the replacement of two hydroxyls by camphene residues thus $\begin{matrix} O=P\langle^{C_{10}H_{15}}_{OH} \\ O \\ O=P\langle^{OH}_{C_{10}H_{15}} \end{matrix}$, and the *β*- as derived from orthophosphoric acid as above. If the latter explanation is correct, the formula of the acid and its sodium salt would be respectively $(C_{10}H_{15}PO_2H)_2O+2H_2O$ and $(C_{10}H_{15}PO_2Na)_2O+9H_2O$, and further, we should expect that the *β*-acid and its salts should be convertible into the *a*-acid and its salts, which has so far proved not to be the case.

If both acids are regarded as derived from orthophosphoric acid, the cause of the isomerism must be looked for in the different positions which the phosphoric grouping occupies in each case with respect to the camphene nucleus.

In view of the investigations of Gamgee and his pupils on the physiological action of the ortho-phosphate and pyro-phosphate of soda, it was thought possible that an investigation of the physiological action of the *a*- and *β*-sodium camphene-phosphonates might throw light on these points, and in any case would yield possibly interesting information as to the effect of the introduction of a camphene group in

the molecule, the new substance being as readily soluble as that from which it was derived.

The following observations deal with some of the physiological properties of these bodies:—

The chemical portion of the research is the work of Gardner, whilst the physiological side was, in the early part of 1909, commenced by the late Professor Gamgee. On Professor Gamgee's death, after a few preliminary observations, the physiological work was undertaken by Symes.

The sodium salts of the two acids were employed throughout the work, and, since Ringer's solution was inadmissible as a solvent—owing to the precipitability of the α-acid by salts of calcium—were invariably dissolved in distilled water containing 0·6 or 0·9 per cent. of sodium chloride.

Three sets of observations were made with these salts:—

1. By intravascular injection, on the blood pressure and respiratory movements of cats, under full anaesthesia.
2. By perfusion on the surviving heart of the frog.
3. By hypodermic injection, on rodents and frogs, in the absence of anaesthetic.

II. Observations on the Blood Pressure and Respiratory Movements

As already stated, these were made on cats. The animals were fully anaesthetised, and maintained in a state of complete anaesthesia by ether.

Tracings of the blood pressure were taken from the carotid artery, whilst the drug was injected into the saphenous vein.

The possibility that the salts of the α- and β-acids were, in solution, respectively pyro- and ortho-phosphonates, led at the outset to their comparison with pyro- and ortho-phosphate of sodium. The physiological action of these latter salts had been studied by Gamgee[1] in collaboration with Priestley and Larmuth, who described the pyrophosphate as being toxic to the central nervous system and to the heart, and the ortho-phosphate as inert. We have repeated and confirmed these observations.

1. Gamgee, Priestley and Larmuth, *Journal of Anatomy and Physiology*, Vol. XI, London, pp. 255-272 and 273-284, 1876-77.

Pyrophosphate of sodium

The effect of a small intravenous injection of sodium pyrophosphate (20-30 mgm. of $Na_4P_2O_7$) is to produce a temporary fall in arterial pressure without any obvious effect on the respiration. A larger dose (50-60 mgm.) produces a greater temporary fall of blood pressure with or without slowing or weakening of the respiratory movements. A lethal dose, 80 mgm. and upwards, kills either by immediate arrest of the heart,[1] followed by failure of respiration, or by immediate cessation of respiration followed by temporary recovery from the initial fall of blood pressure. As might be expected, it is the more rapid injections that favour cardiac death.

Doses of sodium orthophosphate (Na_2HPO_4) equimolecular to the above produce little or no recognisable effect either on the blood pressure or on the respiratory movements.

Solutions of $Na_4P_2O_7$ are markedly alkaline, and some of their poisonous action is due to their alkalinity. We find, e.g., that addition of hydrochloric acid up to a certain point diminishes their effect on the blood pressure, and that intravenous injections of sodium hydrate produce on the blood pressure effects closely resembling those of the pyrophosphate.

But alkalinity is not the only factor in the toxicity of these salts, for their solutions neutralised with HCl to litmus, to phenol phthalein, or to methyl orange are still poisonous, in spite of the fact that a portion of the pyrophosphate undergoes hydrolysis in the course of the neutralisation.

Orthophosphate of sodium

Gamgee, Priestley and Larmuth, employing Na_3PO_4, found that three injections, each = 115 mgm. of this salt, produced no material change in the blood pressure or pulse rate of a rabbit weighing 1·8 kilos.

Since Na_3PO_4 must, in the blood, be transformed largely if not wholly into Na_2HPO_4, we have preferred to use the latter salt. On a 3 kilo. cat, 142 mgm. of Na_2HPO_4 slightly raised the carotid pressure, and slowed respiration from 22 per minute to 15 per minute, whilst 284 mgm. led to a temporary fall of 40-50 mm. Hg in blood pressure with reduction of respiration from 18 per minute to 12 per minute, and 568 mgm. on the same cat produced no greater effect. These three doses

1. On one occasion after a cardiac death a clot was found in the right heart.

are, in molecules, respectively equivalent to 164, 328 and 656 mgm. of Na_3PO_4, and to 266, 532 and 1064 mgm. of sodium pyrophosphate.

In large doses (500 mgm. and upwards), of sufficient concentration (M/2 per litre), Na_2HPO_4 leads to marked dyspnoea, commencing sometimes as late as two (or more) minutes after injection and lasting about thirty seconds. A similar effect is produced by equally hypertonic injections of other relatively inert drugs, for example, by sodium sulphate.

Sodium a-Camphene-phosphonate

Like pyrophosphate of sodium, this salt lowers blood pressure, but gram for gram, to a much smaller extent. Its effect on respiration, except in large doses, is practically zero.

In our first experiment, which was done with Professor Gamgee, the results were as follows:—

TABLE I.—Cat, 2·9 kilos, 16 March, 1909

Time	Intravenous Dose	Mean Carotid pressure	
		Before injection	After injection
3.27 p.m.	58 mgm. (2.5 c.c.)	166 mm. Hg	150 mm. Hg
3.30 „	58 „ (2·5 c.c.)	154 „	150 „
3·35 „	116 „ (5 c.c.)	160 „	140 „
4.2 „	249 „ (5 c.c.)	160 „	128 „
4·7 „	249 „ (5 c.c.)	146 „	120 „
4·46 „	131 „ (5 c.c.)	132 „	118 „

As would be expected, the effects of the larger doses were greater in duration as well as in degree. There was no noticeable effect on respiration.

The three following tables show the comparative activity of the *a*-camphene-phosphonate, and pyrophosphate of sodium.

TABLE II. Cat A, 2·3 kilos, 24 September, 1909

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration
		Before injection	After injection	
3.3 p.m.	Phosphonate 229 mg. (5 c.c.)	150 mm. Hg	160 mm. Hg	Unaltered
3.7 p.m.	Pyrophosphate 135 mg. (5 c.c.)	150 mm. Hg	50 mm. Hg	First quickened then slowed
3.10 p.m.	Phosphonate 229 mg. (5 c.c.)	130 mm. Hg	110 mm. Hg	Unaltered
3.13 p.m.	Pyrophosphate 135 mg. (5 c.c.)	125 mm. Hg	35 mm. Hg	First quickened, then arrested 30 sec., then recovered.

On the supposition that the camphene compound is a pyrophosphonate, these doses are equimolecular.

TABLE III. Cat B, 2·6 kilos, 24 September, 1909.

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration
		Before injection	After injection	
4.4 p.m.	Phosphonate 389 mgm. (8·5 c.c.)	160 mm. Hg	140 mm. Hg	Slightly slowed
4.9 p.m.	Pyrophosphate 108 mgm. (4 c.c.)	160 mm. Hg	40 mm. Hg	Frequency halved
4.50 p.m.	Phosphonate 458 mgm. (5 c.c.)	150 mm. Hg	110 mm. Hg	Slightly slowed
4.52 p.m.	Pyrophosphate 135 mgm. (5 c.c.)	150 mm. Hg	Death (heart failure)	

On the supposition that the camphene compound is a pyrophosphonate, each phosphonate dose is, in molecules, double that of the pyrophosphate immediately following it.

TABLE IV.—Cat, 2·7 kilos, 17 November, 1909

Time	Intravenous dose	MEAN CAROTID PRESSURE		Respiration	
		Before Injection	After Injection	Before injection	After injection
3.57 p.m.	Phosphonate 500 mgm. (5 c.c.)	140 mm. Hg	110 mm. Hg	26 per min.	20 per min.
4.6 p.m.	Phosphonate 1,000 mgm. (15 c.c.)	140 mm. Hg	96 mm.Hg	33 per min.	20 per min.
5.5 p.m.	Pyrophosphate 135 mgm. (5 c.c.)	114 mm. Hg	Death (heart failure)		

On the supposition that the camphene compound is a pyrophosphonate, the dose of $Na_4P_2O_7$ is equimolecular with 229 mgm. of the sodium *α*-camphene-phosphonate.

Sodium β-camphene-phosphonate

Intravenous injection of this salt has, on cats, effects very similar to those of the corresponding *α*-compound, as is seen on comparing the following table with Tables I to IV:—

TABLE V

Intravenous injections of sodium β-camphene phosphonate

Weight of cat	Dose	MEAN ARTERIAL PRESSURE		RESPIRATION	
		Before injection	After injection	Before injection	After injection
2·9 kilos. (16 Mar., 1909)	119 mgm. (5 c.c.)	178 mm. Hg	170 mm. Hg	36 per min.	37 per min.
3·0 kilos.	238 mgm. (5 c.c.)	180 mm. Hg	180 mm. Hg	37 per min.	28 per min.
(24 Sept., 1909)	476 mgm. (10 c.c.)	180 mm. Hg	170 mm. Hg	20 per min.	11 per min.
3·4 kilos.	500 mgm. (5 c.c.)	166 mm. Hg	126 mm. Hg	30 per min.	33 per min.
(21 Feb., 1910)	1000 mgm. (10 c.c.)	162 mm. Hg	116 mm. Hg	25 per min.	26 per min.

In short, the camphene-phosphonates of sodium are, on the anaesthetised cat, relatively inert bodies. Their effects are similar and, gram for gram, approximately equal. Both, in sufficient (intravenous) dose, lower the blood pressure, and both tend to reduce the frequency of respiration.

Both are rapidly excreted in the urine, in which they are recognisable by their reaction with bromine water, about ten minutes after an injection.

III. Observations on the Surviving Heart

These were made on decapitated and pithed frogs. The heart was fed through the inferior vena cava or through the hepatic vein, from Marriotte flasks, with a 'head' of about 2 cm. of water. The ventricle emptied itself into the aorta, the fluid circulating through the capillaries[1] and escaping from the abdominal veins which were freely opened on the distal side of the feeding cannula. With a feeble heart it was sometimes necessary to sever one of the aortic arches.

The contractions of the heart were recorded by a light, partially counterpoised lever, connected by a thread to the apex of the ventricle.

For the reason already given, the solvent of the phosphates and phosphonates was 0·6 per cent. solution of sodium chloride in distilled water, which served also as the 'normal saline' solution against which the bodies under examination were tested.

With this 'normal' solution, oxygenated, and employed as above, a heart is usually available for experiment for upwards of one hour.

Action of sodium pyro- and ortho-phosphate

On the frog heart, administered as above, sodium pyrophosphate is markedly active. A solution containing 0·26 per cent. (M/100) may reduce the ventricle almost to standstill within one minute. As described by Gamgee and his pupils, the arrest is usually in systole,[2] but diastolic arrest is not uncommon. The effect of the salt can, however, be largely washed out by the normal saline. Stronger solutions act more energetically, and their effect is more permanent. A 2·6 per cent. solution (M/10) has produced permanent arrest of both chambers.

1. Apart from escape from severed arteries.
2. As with other alkaline solutions.

Sodium orthophosphate (Na_2HPO_4) is far less active. A solution containing 1·4 per cent. (M/10) reduces somewhat the force and frequency of the ventricular beat, but has much less effect than a M/100 (0·26 per cent.) solution of the pyrophosphate.

Less concentrated solutions of the orthophosphate, e.g. 0·35 per cent. (M/40) have often a restorative effect on a flagging heart, though on hearts that are beating vigorously they have usually, during perfusion, the opposite tendency.

As pointed out above, some, but not all, of the effect of sodium pyrophosphate on the anaesthetised cat is due to the alkalinity of its solutions. The same appears to be true for the surviving heart of the frog, since M/100 solutions of the pyrophosphate are more active than M/10 solutions of Na_2HPO_4, and are not more alkaline than the latter. Owing to the gradual hydrolysis of the former salt, when its solutions are neutralised, it was not possible to investigate this point more closely.

Action of the camphene-phosphonates of sodium

The aqueous solutions of these bodies are neutral.

Gram for gram, they have on the frog's heart, as on the respiration and on the blood pressure of the cat, similar and approximately equal effects.

Dilute solutions (0·4—0·6 per cent.) of either of them first increase then slightly reduce the frequency, and usually also the amplitude, of the beat. Later the amplitude is usually increased, the frequency remaining (sometimes considerably) reduced. Weak saline solutions of camphene produce similar effects.

Solutions ten times as concentrated (4 to 6 per cent.) have failed to arrest the beat within six minutes, beyond which time lack of material has made it impossible to follow their effects.

The pyro-form, which is unquestionably assumed by the *a*-salt when heated, is either lost, on solution in normal saline, or (if retained) is not associated with the enhanced physiological activity that follows heating orthophosphate of sodium.

Regarding both of the phosphonates as ortho- in form, they are, in the frog's heart, molecule for molecule, more active than sodium orthophosphate, much less active than the pyrophosphate of the same metal.

IV. Effect of Subcutaneous Injections of the Phosphonates

These observations were made on frogs, rats and rabbits without anaesthesia. The *a*-phosphonate appeared to be the more active, but neither of them was acutely poisonous.

On frogs their effect closely resembles that of camphene. When inserted beneath the dorsal skin, this substance produces excitement followed by increasing inco-ordination and narcosis. Injections of solutions of the camphene-phosphonates lead more rapidly to the same effects, and doses of 40 mgm. of the *a*-compound have proved fatal within one hour.

On rats and rabbits camphene has little or no obvious effect, whether given by inhalation, or by subcutaneous injection of a solution in cod liver oil, or in ether.

The phosphonates are more active, producing effects similar to those observed on the frog.

The initial effect is excitement, accompanied by congestion of the ears, lips, genitals and pads of the feet. With sufficiently large doses this is followed by narcosis, and occasionally by death.

With both phosphonates the effects are of the same kind, but, as mentioned above, those of the *a*-compound seem the more pronounced, though doses of 1 mgm. per gram body weight are not invariably fatal.

V. Summary and Conclusions

It appears from the foregoing observations that the *a*- and *β*-camphene-phosphonates of sodium differ little from one another in physiological effects or in activity.

Both are far less active than the pyrophosphate, but distinctly more active than the orthophosphate of sodium.

Tested by injection, *in vivo*, they have similar and equal effects on anaesthetised cats. In the absence of anaesthesia, on rodents and on frogs, the *a*-phosphonate is somewhat the more active. As they have not been compared on anaesthetised rodents, or on cats in the absence of anaesthesia, it is not possible to decide whether this difference is due to the anaesthetic or to the animal.

Their effects appear to be chiefly on the central nervous system, and their comparative inertness is doubtless in part due to rapid excretion.

On the frog, and on the surviving heart of the frog, their action closely resembles that of camphene. On the warm-blooded animals the action of camphene is so much less than that of the camphene-phosphonates that comparison is difficult. Such effects, however, as are produced by camphene resemble those of minute doses of the phosphonates.

The small difference between their physiological effects, and the general resemblance of these to those of camphene, suggests that the first of the two possibilities, with regard to their constitution, quoted in the introduction, is the more probable,—namely, that they are isomeric derivatives of orthophosphoric acid, and that the pyro-form which is readily assumed by one of them (α-) on heating is as readily lost when the dried salt is re-dissolved in water.

ON THE PRESENCE OF ALLANTOIN IN CERTAIN FOODS

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(Received January 13th, 1911)

Recent investigators who have worked with reliable methods are all agreed that allantoin is a constant constituent of normal human urine when the diet of the subject is of a mixed character. The amount of this allantoin is quite small, not exceeding 15 mgm. a day, and the quantity is not increased when the food contains a large amount of purin substances,[1] or in diseases such as leukaemia.[2, 3] Several observers,[1, 4, 5, 6] have shown also that allantoin, given by the mouth to men, can be recovered to a considerable extent in the urine. It is possible, therefore, that the allantoin found in human urine is not derived from the breakdown of uric acid in the body, but is contained preformed in the food and is excreted unchanged. Schittenhelm and Wiener[1] failed to find allantoin in the urine of a person on a diet composed of milk and vegetables, but Wiechowski[2] found 13-14 mgm. per diem in the urine of two persons on a 'purin-free' diet, and Ascher[3] found its presence in the urine of an individual during starvation. This person, however, starved for 28 hours only, and in the urine which he passed during the last 24 hours only 1 mgm. of allantoin was found; this might have been derived from the food taken on the preceding day, and cannot be accepted as conclusive proof of the endogenous origin of allantoin in man.

It has not been proved that the amount of purin substances present in a food bears any relation to the quantity of allantoin which that food contains. Allantoin has, however, been frequently found in the vegetable kingdom—in the leaf buds of *Platanus orientalis*,[7] of *Acer pseudoplatanus*, of *Acer campestre*, and in the bark of *Aesculus hippocastanum* and of *Acer pseudoplatanus*.[8] It has been found also in the embryos of wheat separated in the process of milling,[9] and in beet juice,[10] but it has not, so far as I know, been looked for in the ordinary foods.

So-called purin-free diet is not generally absolutely purin free, but contains such small traces of purin substances that the uric acid

produced therefrom in metabolism is negligible by comparison with the relatively large amount which has an endogenous origin: thus milk, according to Burian and Schur,[11] contains 0·004—0·006 gram of purin nitrogen per litre, while according to Bessau,[12] it is completely purin free. Most vegetable products also, though not all, contain traces of purin substances, but in most of these the quantity is quite negligible. In the case of allantoin the matter is quite different, for the amount of that substance found in human urine is so small that even traces, if present in a number of foods, may account for all the allantoin excreted.

From this point of view I have looked for allantoin in milk, white bread, eggs, French beans, green peas, bananas and rhubarb, and have found it present in some of these foods in quantities quite sufficient to account for the 13-14 mgm. per diem excreted on a 'purin-free' diet. It was found that milk sometimes contained as much as 0·019 gram per litre, but the quantity is probably very variable; white bread contained 0·006 gram per kilogram, while eggs were completely allantoin free. French beans, which, according to Bessau,[12] contain only 0·0021 gram per cent. of purin nitrogen, were found to contain 0·0287 gram per cent. of allantoin (equivalent to 0·009 gram per cent. of nitrogen), while green peas containing a large quantity of purin nitrogen (0·0274 gram per cent.) contain only 0·00026 gram per cent. of allantoin (equivalent to 0·00009 gram per cent. of nitrogen); bananas and rhubarb were completely allantoin free. Foods, therefore, containing little or no purin substances, may contain relatively large quantities of allantoin, while foods, such as green peas, containing much purin may contain mere traces of allantoin. *The purin content of a food is, in fact, no guide to its allantoin content.* The difficulty of completely extracting and isolating such a substance as allantoin in the pure state from large amounts of foods makes it very probable that the above figures do not represent very closely the amounts actually present, which are almost certainly much greater in the case of bread, French beans, and peas.

If, therefore, allantoin is not destroyed in the human organism, and the amount excreted on a purin-free diet can be accounted for as occurring preformed in the food, and if the amount excreted cannot be increased by the administration of large quantities of uric acid precursors, then the conclusion seems to be justified that uric acid is not converted into allantoin in the course of metabolism in man: but the facts herein referred to leave untouched the much discussed question as to whether man has the power to destroy uric acid in any other way.

Experimental Part

Milk. Three litres of milk, from which the fat had been removed as far as possible by centrifugalisation, were boiled and acidified with acetic acid; the precipitated casein was filtered off, broken up into fine particles, again boiled for a few minutes with two litres of water, and again filtered off on a large 'nutsche.' The two filtrates were added together and completely precipitated by basic lead acetate; the filtrate from this was quite clear, and, after removing excess of lead by sulphuretted hydrogen, and this by a current of air, it was neutralised with sodium hydrate and 20 per cent. solution of mercuric nitrate added till a filtered sample gave a precipitate at once with very dilute allantoin solution; the fluid was then again neutralised and left to stand over night. The precipitate was filtered off through a folded filter, well washed with cold distilled water, suspended in about 300 c.c. of distilled water and decomposed with sulphuretted hydrogen, the mercury sulphide was filtered off and well washed, the filtrate and wash water (500 c.c. in all) were acidified with acetic acid and concentrated to about 250 c.c. on the water bath. This solution was then treated by Wiechowski's method for isolating allantoin, i.e., it was precipitated by phosphotungstic acid in sulphuric acid solution, the excess of phosphotungstic acid and the sulphuric acid removed from the filtrate by agitation with lead oxide till neutral, and again precipitated with basic lead acetate.

After removal of the excess of lead by sulphuretted hydrogen, small samples of the fluid were tested with phosphotungstic acid, with basic lead acetate, and with silver acetate, none of which gave any precipitate. The fluid was then concentrated to about 150 c.c., neutralised exactly with sodium hydrate, and excess of Wiechowski's mercury acetate reagent added; a white flocculent precipitate came down at once; this was left to stand over night. A sample of the clear fluid gave a precipitate at once with a very dilute solution of allantoin. The precipitate was filtered off, suspended in 200 c.c. of distilled water, boiled and decomposed hot with sulphuretted hydrogen; it was then evaporated on the water bath to dryness, the residue extracted with hot water, the mercury sulphide filtered off, and the filtrate evaporated to dryness on the water bath. There was left a deeply pigmented yellowish orange mass; the pigment could not be removed by alcohol, ether, or chloroform, but by careful washing with very small quantities of cold water, and once re-crystallisation from hot water, an almost white

crystalline substance was obtained. This weighed 0·013 gram, had the crystalline form of allantoin, was difficultly soluble in cold water, almost insoluble in alcohol, its aqueous solution was not precipitated by phosphotungstic acid or basic lead acetate. It was not precipitated by silver nitrate alone, but came down at once as a flocculent precipitate on further adding a trace of ammonium hydrate, readily dissolving in excess of the latter, and again coming down on addition of more silver nitrate. It gave the glyoxylic reaction and melted with decomposition at 231° C.; when mixed with an equal quantity of pure allantoin (Merck) the melting point remained the same.

In this experiment the pigment, probably derived from the cow's food, was so difficult to remove, the use of charcoal being inadmissible, that a considerable quantity of allantoin was lost in obtaining it pure.

In a second experiment, three and a half litres of milk were treated in exactly the same way. The final residue was almost colourless; by washing with about ½ c.c. of water and re-crystallising once it was obtained quite white. It had the characters previously described, and melted with decomposition at 232° C.; mixed with an equal quantity of Merck's allantoin the melting point was unchanged. The amount obtained in the pure state was 0·0665 gram, equivalent to 0·019 gram per litre of milk.

Bread. 1100 grams of white bread were cut up into slices and dried in a hot air oven; the dry weight was 740 grams. It was then finely powdered and ground up in a mill to a fine flour. This was boiled with several litres of water; it swelled up into a semi-gelatinous mass, from which no fluid could be removed; it was therefore diluted with water to a thin gruel, and filtered through a large felt jelly bag; the residue was squeezed out as far as possible, and was then again mixed with a large quantity of water and again filtered and squeezed out. The combined filtrates (about 8 litres) were precipitated with basic lead acetate, and the clear filtrate so obtained treated in the same way as described for milk. On evaporating the fluid obtained by decomposition of the mercury acetate precipitate, a gummy residue was left behind, which at first showed no signs of crystallising, but on standing for 24 hours a crystalline substance separated out. This was isolated by washing with a very small quantity of cold water and re-crystallising from a minimal quantity of hot water. 0·0036 gram of a white substance having the crystalline form of allantoin was obtained. Its solutions gave the glyoxylic reaction, and were precipitated by silver nitrate in the presence

of a trace of ammonia, the precipitate being soluble in excess of the latter. It melted with decomposition at 229·5° C.

In a second experiment 2400 grams of white bread were dried and ground up as before; the dried weight was 1618 grams. In order to avoid the swelling up produced by boiling, this was extracted cold for four days with 6 litres of water, toluol being added to prevent bacterial action; it was then filtered through a felt jelly bag, the residue mixed with a further 4 litres of water, and again filtered, the residue being again treated in a similar way. The combined filtrates were precipitated with basic lead acetate, and the clear filtrate obtained treated as above. As in the first experiment, a gummy residue was left, from which crystals slowly separated out; they were isolated in the same manner. 0·0148 gram of a white substance, having all the characters already given for allantoin, was obtained. It melted with decomposition at 231° C., and when mixed with an equal quantity of Merck's allantoin the melting point remained unchanged.

This represents 0·006 gram of allantoin per kilogram of bread, but the difficulty of complete extraction of the bread, and the isolation of the pure substance from the gummy residue obtained at the end, make it very probable that this figure does not represent the whole of the allantoin present in bread.

Eggs. Twelve eggs, weight without shells 510 grams, were well beaten up with three litres of water, acidified with acetic acid, boiled and filtered; the coagulum was broken up into fine particles and re-extracted by boiling with two litres of water, and was again filtered off on a large 'nutsche.' The combined filtrates were concentrated on the water bath to 400 c.c., filtered, and the filtrate precipitated successively by phosphotungstic acid, basic lead acetate, and silver acetate, exactly as in Wiechowski's method; the filtrate from the silver acetate, after removal of excess of silver by sulphuretted hydrogen, was concentrated on the water bath to 150 c.c., and Wiechowski's mercury acetate reagent added. At first no precipitate came down, but on standing over night a very faint precipitate appeared; the filtrate from this gave a precipitate at once with a dilute solution of allantoin.

The precipitate, when decomposed in the usual way, gave only a slight gummy residue, from which no allantoin could be obtained, nor did it when dissolved in water give the glyoxylic reaction.

Peas. 4500 grams of fresh green peas were finely chopped up in a mincing machine, and extracted for two days with two litres of 98 per

cent. alcohol; this was then filtered off through a jelly bag and the residue squeezed dry in a press; the residue was again extracted for two days with 98 per cent. alcohol, filtered, and pressed as dry as possible. The residue was then extracted twice with petroleum ether to remove fat, and dried in the air; it was then ground up in a mill to a fine flour and extracted with several litres of boiling water, filtered through a jelly bag, and squeezed dry in a press, the residue being again extracted with boiling water, filtered, and pressed dry. The alcoholic extracts were mixed, the alcohol driven off on the water bath, and the remainder added to the two watery extracts. The whole fluid was then precipitated by basic lead acetate, thus obtaining a perfectly clear filtrate, which was afterwards treated in exactly the same manner as described for milk, except that previous to the final precipitation by mercury acetate the fluid was not concentrated below 250 c.c., as it was thought that more allantoin might be present in green vegetables. A gummy residue was obtained from the decomposition of the mercury acetate precipitate, from which, on standing, crystals separated out. By cautiously washing with a small quantity of water these were obtained almost quite white, but were found to consist of two different forms of crystals, one being large columnar crystals, the other prismatic, like allantoin. By fractional crystallisation the allantoin, which was much the less soluble, was obtained pure. The weight isolated was 0·0117 gram; it had all the characters already given for allantoin. The melting point was 230° C. with decomposition, and when mixed with an equal part of Merck's allantoin the melting point remained unchanged.

French beans. 4350 grams of fresh French beans were treated in the same way as that described for peas, the fluid which separated during the mincing being carefully collected and added to the other extracts. The final precipitate with mercury acetate was very voluminous; when it was decomposed and the filtrate evaporated, a comparatively large mass of crystals began to separate out. These were uniform in character, and had the crystalline form of allantoin, but the mother liquor from these crystals gave, an evaporation to dryness, more crystals, some of which resembled allantoin and some did not, being very much larger and columnar. The two substances were separated by fractional crystallisation, allantoin being much the more insoluble.

The total amount of allantoin isolated was 1·2495 grams; this had all the characters already given for allantoin. Its melting point with decomposition was 230° C., which remained unchanged when mixed with

an equal quantity of Merck's allantoin. After one re-crystallisation, 0·1614 gram of the substance was used for nitrogen analysis by Kjeldahl's method, 40·8 c.c. of decinormal acid were required, equivalent to a nitrogen content of 35·39 per cent., the theoretical percentage of nitrogen in allantoin being 35·44 per cent.

Bananas. 445 grams of ripe bananas were mashed up and boiled with one litre of water, and filtered through linen. The filtrate, after precipitation with basic lead acetate, and removal of excess of lead from the filtrate by sulphuretted hydrogen, was evaporated on the water bath to about 200 c.c.; this was then treated by Wiechowski's method. The mercury acetate reagent gave a voluminous precipitate, but no allantoin could be isolated from it, the crystalline substance obtained having none of the characters of allantoin.

Rhubarb. 4350 grams of peeled rhubarb were mashed up and squeezed out in a press, the residue was boiled with water and again squeezed out dry; the filtrate was added to the fluid already obtained, and the whole precipitated by basic lead acetate. The filtrate, after removal of excess of lead by sulphuretted hydrogen, was concentrated in vacuo to about 400 c.c.; it was then treated by Wiechowski's method. Only a very faint precipitate came down with the mercury acetate reagent, from which no allantoin could be isolated.

Conclusions

The whole quantity of allantoin excreted by man on a milk and vegetable diet may be derived directly from the food. Milk, white bread, French beans, green peas, all contain small quantities of allantoin, while none could be isolated from eggs, bananas, or rhubarb.

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THE EFFECT OF CHLOROFORM ON THE INTERMEDIATE PROTEIN METABOLISM OF THE DOG

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Communicated by Professor D. Noël Paton

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The administration of chloroform has a very marked effect on the chemical changes in the body. It causes a considerable acceleration in the protein metabolism, as evidenced by the increased excretion of nitrogen. It has a marked action upon the liver cells outside the body,[1] and after its administration by various channels to the living animal, Dr. Clark has shown that the liver cells show histologically very great degenerative changes.[2] Dr. Noël Paton[3] carried out a series of experiments to determine the effects of chloroform on the metabolism with especial consideration of the manner in which the power of the liver to elaborate urea was affected. In both these papers the literature of the subject is very fully considered.

The immediate result found was a considerable fall in the proportion of nitrogen in the form of urea, which was followed some days later by a rise in the proportion of nitrogen occurring as ammonia. The proportion of undetermined nitrogen rose with the fall in urea and previously to the rise in ammonia.

From the methods employed by Noël Paton[4] for the determination of the urea nitrogen it appeared probable that some of the undetermined nitrogen might be due to the appearance of amino acids in the urine, possibly along with some other intermediate products of protein metabolism. Since the nature of such intermediate products is a question of considerable interest as throwing light on the course of catabolism, the present detailed examination of the distribution of nitrogen in the urine of dogs after the administration of chloroform was undertaken.

1. Nöel Paton, *Phil. Trans. Roy. Soc.*, Vol. CLXXXV, p. 248.
2. *Proc. Roy. Soc. Edin.*, Vol. XXVIII, 1908.
3. *Proc. Roy. Soc. Edin.*, Vol. XXVIII, 1908.
4. *Loc. cit.*

After my work was nearly completed, Howland and Richards[1] published the results of investigations on the metabolic changes in the case of three dogs, two of which died of delayed chloroform poisoning. They observed the same fall in urea nitrogen and increase in ammonia and undetermined nitrogen. Where delayed poisoning was induced the changes were the same in character, but more marked than when the animals made a normal recovery.

In the present experiments the chloroform was administered by Professor D. Noël Paton, and the conditions were kept exactly similar to those of his previous investigation. The animals were fed on porridge and milk, and on the day of administration food was usually withheld till after the dose had been given. The urine was collected in the metabolic cages used in this laboratory.

If given hypodermically two doses of 5 c.c. were administered on two successive days. If given by the mouth the dose was 25 c.c. chloroform in oil. In most of the experiments the chloroform was injected hypodermically, as, while the effect on the metabolic changes was the same as when given by the mouth, digestive disturbances were less likely to occur.

Methods of Analyses

The *total nitrogen* was determined by Kjeldahl's method.

The *urea nitrogen* was determined by the Mörner-Folin method.

The *ammonia nitrogen* was determined by Folin's method.

The *allantoin nitrogen* was determined by a combination of Folin's and the Mörner-Folin methods for the determination of urea as described by me.[2]

The *amino acid nitrogen*, including hippuric acid nitrogen, was determined by a combination of Bohland's phosphotungstic acid method for the estimation of urea, and Folin's method, also described by me.[3]

Creatine and creatinine nitrogen were determined by Folin's colorimetric method, using a Duboscq colorimeter.

Protein, if present in any quantity, was precipitated by adding an equal volume of 10 per cent. trichloracetic acid in the cold before any of the above estimations were carried out.

1. *Journ. of Exper. Med.*, Vol. XI, 1909.
2. *Bio-Chem. Journ.*, Vol. IV, p. 448, 1909.
3. *Loc. cit.*

The question of the error involved in such a differential method is of importance. In each case control estimations were made, and the difference between the two titrations ranged as a rule from 0 to 0·5 c.c. N/10 acid. Taking the mean of the titrations, this difference corresponds to about ± 2 per cent. of the total nitrogen. In some exceptional cases—not more than five throughout the whole series of experiments—the difference between the two titrations gave rise to a difference of ± 5 in the percentage of total nitrogen. These experiments are noted with an asterisk.

The possibility of such an error would seem to throw some doubt on the results obtained by these methods. In no case, however, did this error occur in the same series of analyses in more than one of the three methods—Bohland, Folin, Mörner-Folin—by which the nitrogen was determined. We may therefore conclude that the error of the method does not vitiate the conclusions drawn.

Sorensen's formol titration method,[1] based upon the principle that the amino group in ammonia and amino acids reacts with formaldehyde, has been elaborated by Henriques[2] for the determination of the amino acid nitrogen.

This method I have used in several instances in this series of experiments as an alternative and check to the method of amino acid estimation above described.

Much work has recently been done on the accuracy of this method. Using it, Henriques,[3] Frey and Gigon,[4] Henriques and Sorensen,[5] Tanzo Yoshida,[6] all found that the urine normally contained a considerable percentage of amino acids.

Malfatti,[7] the details of whose method vary considerably from those of Henriques, finds amino acids to be normally absent, or at most present only in traces.

Oehler,[8] who employed β-naphthaline sulphochloride for the estimation of the amino acids, got similar results. Malfatti, in a later paper,[9] in reviewing the previous work, criticises the use of litmus as an indicator in the first stage of the process.

1. *Biochem. Ztsch.*, VII, 1908.
2. *Ztsch. f. phys. Chem.*, LX, 1909.
3. *Loc. cit.*
4. *Biochem. Ztsch.*, XXII.
5. *Ztsch. f. phys. Chem.*, LXIII.
7. *Ztsch. f. phys. Chem.*, LXI.
6. *Biochem. Ztsch.*, XXIII.
8. *Biochem. Ztsch.*, XXI.
9. *Ztsch. f. phys. Chem.*, LXVI.

Jager[1] also questions the accuracy of the results obtained with litmus as indicator, and, like Malfatti, employs phenol phthalein alone. He discusses[2] the influence of urea on formaldehyde, but with the concentration of urea solution which occurs in the urine he concludes that as employed for the estimation of amino acids in the urine, the method gives, in practice, good results.

Since, however, the method I had devised had proved reliable, and had been used in the earlier experiments of the series, it was employed throughout this investigation.

Results

Experiment I.—Collie dog. Twenty-five c.c. of chloroform in olive oil were given on the morning of the third day. This caused sickness, so the dose was repeated later in the day.

Table I

Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	2·747	0·133	—	—	—	0·137	0	0·108
2	3·567	0·218	2·762	0·041	0·139	0·156	0·006	0·245
3*	2·828	0·12	2·26	0·028	0·118	0·125	0·002	0·175
5	4·488	0·239	3·73	0·088	0·013	0·121	0·050	0·247
6	5·87	0·506	3·826	—	0·795	0·133	0·073	0·537
7	3·382	0·309	2·211	0·045	0·323	0·138	0·042	0·314
8	3·024	0·355	2·102	0·088	0·048	0·109	0·055	0·267
9	2·374	0·219	1·811	0·053	0·011	0·086	0·018	0·176
10	2·974	0·207	2·423	—	—	0·130	0·011	0·117
1	2·632	0·188	2·108	0·067	—	0·105	0	—

Table II

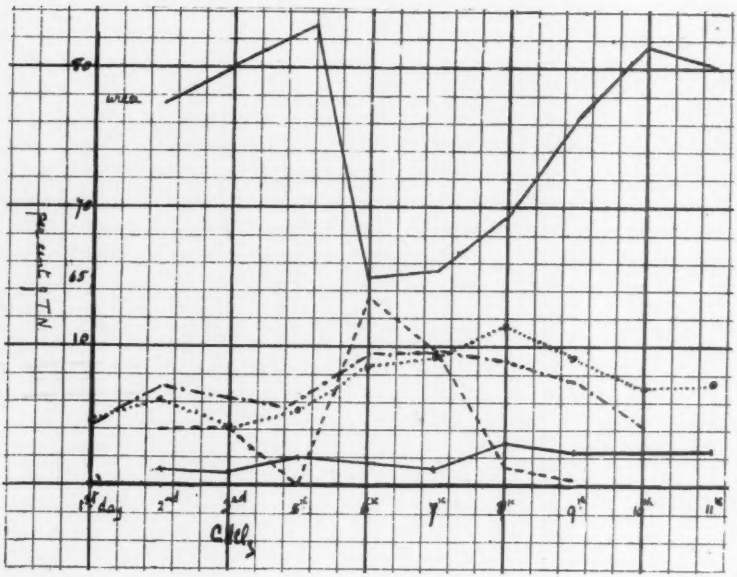
Percentage of Total Nitrogen

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	4·8	—	—	0	4·6	0	4·4
2	6·1	77·4	1·1	3·9	4·3	0·2	7·0
3*	4·2	80·0	0·9	4·1	4·4	0·1	6·3
5	5·3	83·1	1·9	0·2	2·6	1·2	5·7
6	8·6	65·2	—	13·4	2·2	1·3	9·3
7	9·1	†65·4	1·3	9·4	4·0	1·3	9·5
8	11·7	69·5	2·9	1·6	3·6	1·8	8·9
9	9·2	76·3	2·2	0·5	3·6	0·7	7·5
10	6·9	81·5	—	—	4·3	0·4	4·1
11	7·1	80·1	2·5	—	3·9	0	—

1. *Ztsch. f. phys. Chem.*, LXII, LXIV.
2. *Ztsch. f. phys. Chem.*, LXVII.

Throughout the curves, tracings represent as follows:—Urea ———. Ammonia ·····. Allantoin ———. Amino acids – – – – –. Und. N —·—·—·.

EXPERIMENT I *



* The size of these charts is reduced by leaving out the middle part between 10 per cent. and the lowest percentage to which the urea nitrogen fell.

Experiment II.—Brown retriever weighing 17·9 kilos.

Twenty-five c.c. chloroform in oil were given on the morning of the third day, and food was withheld.

The dog took no food on the fourth and fifth days, and on these days there was no urine.

On the third day after the chloroform which was the first urine to be examined, there were a few granular tube casts, some albumin and bile pigments.

Sugar was absent.

There was a trace of protein on the next two days, but no tube casts. Gmelin's test also remained positive.

TABLE III

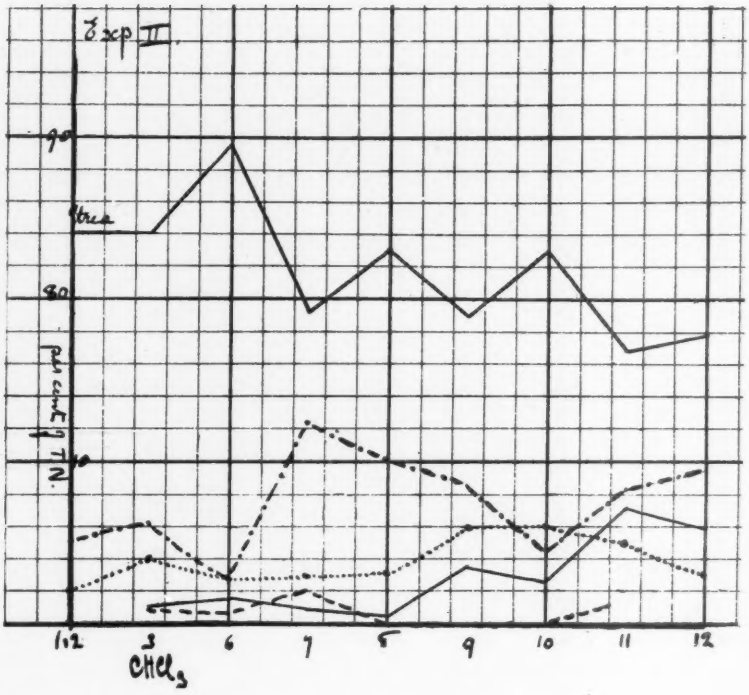
Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1 2	5·346	0·107	4·5	—	—	0·079	0·073	0·282
3*	4·889	0·203	4·128	0·043	0·049	0·135	0·030	0·301
6	8·946	0·257	8·003	0·140	0·067	0·232	0·022	0·225
7	5·2	0·147	4·119	0·049	0·021	0·120	0·010	0·657
8	4·648	0·137	3·867	0·028	0·011	0·136	0·003	0·466
9	4·424	0·262	3·49	0·157	0·004	0·122	0	0·389
10	3·36	0·202	2·808	0·081	0	0·119	0	0·150
11	4·427	0·219	3·4	0·308	0·046	0·131	0·004	0·319
12	4·585	0·140	3·57	0·266	—	0·130	—	0·438

TABLE IV

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1 2	2·0	84·1	—	—	1·4	1·3	5·2
3*	4·1	84·1	1·2	1·0	2·7	0·6	6·3
6	2·8	89·5	1·5	0·8	2·5	0·3	2·6
7	2·8	79·2	0·8	2·1	2·3	0·1	12·7
8	2·9	83·2	0·6	0·2	2·9	0·1	10·1
9	5·9	78·9	3·5	0·1	2·9	0	8·7
10	6·0	83·2	2·7	0	3·5	0	4·6
11	4·9	76·8	7·0	0·9	2·9	0·1	7·4
12	3·0	77·9	5·8	—	2·8	—	9·4

EXPERIMENT II



Experiment III.—The same dog as in *Experiment II.*

During the course of the experiment the animal's weight fell from 19·3 to 17·6 kilos.

Five c.c. chloroform were given hypodermically on the afternoon of the first day, and the same dose again on the morning of the second.

The dog took its food regularly each day.

The urine was very dark on the second day after the chloroform, and showed faint trace of bile pigments on the next two days.

TABLE V

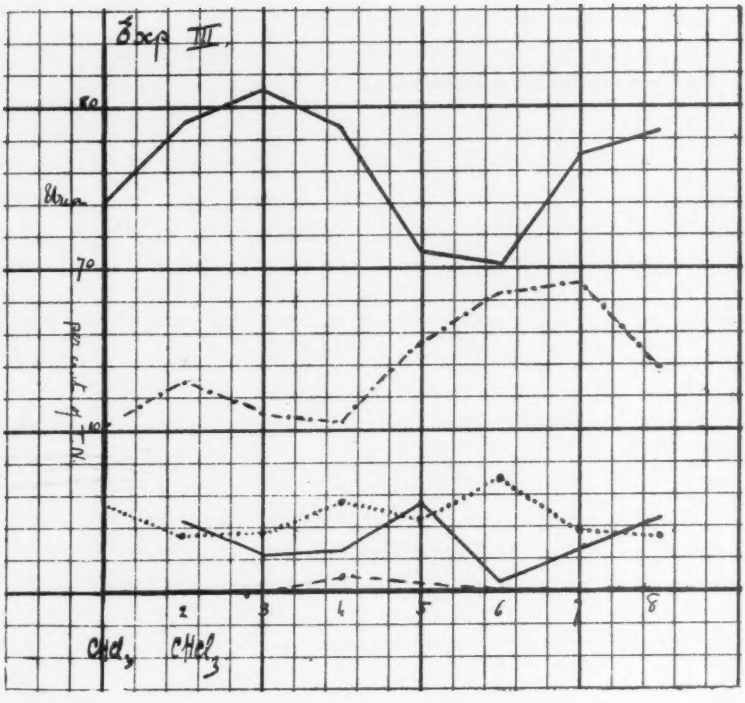
Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	2·94	0·165	2·18	0·189	0·005	0·094	0·006	0·301
2*	4·55	0·160	3·585	0·203	—	0·114	0·020	0·58
3	5·705	0·213	4·634	0·137	—	0·115	0·008	0·617
4	5·355	0·302	4·213	0·147	0·036	0·116	0	0·541
5	3·815	0·171	2·699	0·218	—	0·115	0·003	0·589
6	3·724	0·271	2·613	0·028	0·012	0·098	0·013	0·689
7	3·808	0·148	2·932	—	—	0·108	0·001	0·718
8	4·06	0·141	3·191	0·173	—	0·116	0·002	0·555

TABLE VI

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	5·6	74·1	—	0·1	3·1	0·2	10·5
2*	3·5	78·8	4·4	—	2·5	0·4	12·8
3	3·7	81·2	2·4	—	2·0	0·1	10·9
4	5·6	78·7	2·7	0·8	2·1	0	10·3
5	4·4	70·9	5·6	—	3·0	0·1	15·6
6	7·2	70·2	0·7	0·3	2·6	0·3	18·7
7	3·8	77·0	—	—	2·8	0	19·0
8	3·4	78·6	4·6	—	2·8	0·1	13·8

EXPERIMENT III



Experiment IV.—The same dog as in *Experiment I.*

During the experiment the animal's weight rose from 14·6 to 15·4 kilos.

Five c.c. chloroform were given hypodermically on the afternoon of the second day, and repeated on the morning of the next.

Protein was present in small amount on the sixth and seventh days.

A trace of bile pigments was observable on the fifth, sixth and seventh days.

There was no reduction of Fehling's solution.

TABLE VII

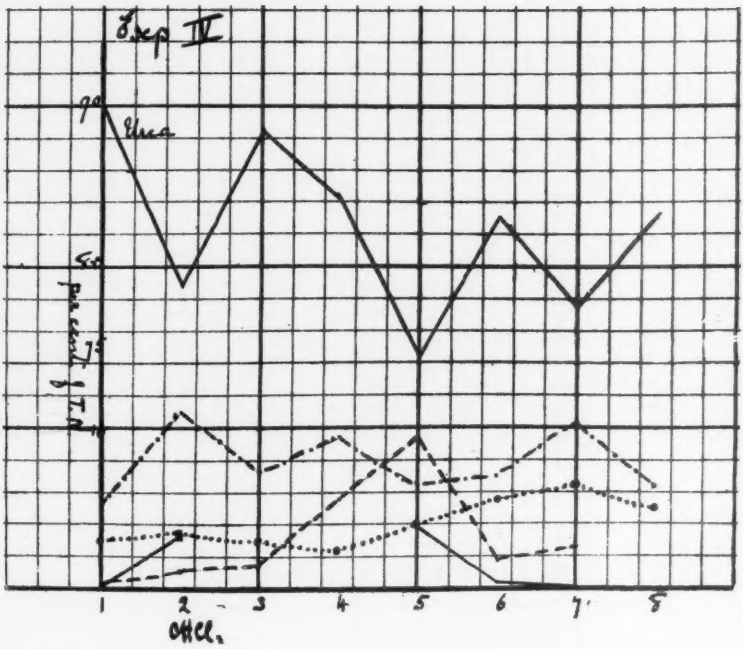
Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	5·32	0·151	4·784	0	0·011	0·103	0	0·278
2*	4 34	0 148	3 422	0 154	0·05	0 086	0	0 48
3	5·04	0·151	4·469	—	0·081	0·109	0	0·37
4	7·42	0·190	6·25	—	0·414	0·126	0	0·706
5	6·09	0·246	4·531	0·235	0·339	0·092	0·035	0·39
6	6·23	0·353	5·177	0·042	0·115	0·119	0	0·424
7	4·312	0·289	3·351	0·011	0·110	0·108	0	0·443
8	3·948	0·193	3·279	—	—	0·093	0·010	0·218

TABLE VIII

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1	2·8	89·9	0	0·2	1·8	0	5·4
2*	3·4	78·8	3·6	1·1	1·9	0	11·2
3	3·0	88·7	—	1·6	2·2	0	7·3
4	2·5	84·2	—	5·5	1·7	0	9·6
5	4·0	74·4	3·8	9·3	1·5	0·5	6·5
6	5·6	83·1	0·7	1·8	1·9	0	6·9
7	6·7	77·7	0·2	2·6	2·5	0	10·3
8	4·8	83·1	—	—	2·3	0·3	5·7

EXPERIMENT IV



Experiment V.—Dog. Whippet weighing 23 kilos.

The animal received 5 c.c. chloroform hypodermically on the first and second days of the experiment.

The urine of the second day contained vomited matter and faeces and therefore was not examined.

The dog took no food till the fifth day.

Protein was present from the third day till on the seventh a marked cloud on boiling with acetic acid was obtained.

The fourth day showed a slight reduction of Fehling's solution.

On the seventh day there was an abundance of granular cells but no tube casts. On this day the urine showed Pettenkofer's test for bile acids distinctly.

TABLE IX

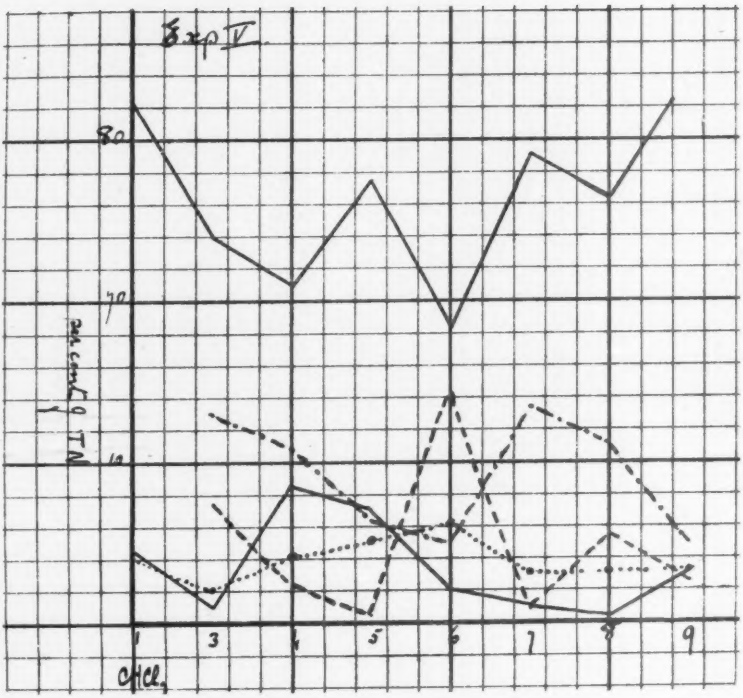
Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	4·091	0·172	3·38	0·182	—	0·184	0·007	—
3	16·432	0·340	12·155	0·189	1·232	0·311	0·095	2·11
4	7·665	0·315	5·46	0·651	0·192	0·165	0·060	0·822
5	5·365	0·276	4·171	0·375	0·081	0·190	0	0·332
6	6·412	0·419	4·397	0·134	0·924	0·200	0·011	0·327
7	8·669	0·242	6·864	0·091	0·099	0·183	0·052	1·269
8	6·783	0·215	5·192	0·044	0·384	0·195	0·008	0·932
9	9·975	0·302	8·238	0·392	0·269	0·289	0·005	0·764

TABLE X

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	4·2	82·6	4·4	—	4·4	0·2	—
3	2·0	†74·0	1·2	7·4	1·8	0·6	13·0
4	4·1	71·2	8·5	2·5	2·1	0·8	10·8
5	5·1	†77·7	7·0	0·4	3·5	0	6·3
6	6·5	68·6	2·0	14·5	3·1	0·1	5·2
7	2·7	†79·2	1·1	1·0	2·1	0·6	13·3
8	3·1	76·6	0·6	5·6	2·8	0·1	11·2
9	3·0	82·6	3·9	2·6	2·8	0·1	5·1

EXPERIMENT V



Experiment VI.—Black retriever weighing 17·6 kilos.

4 c.c. chloroform were given hypodermically on the first and second days.

There was a small amount of protein present on the fourth and fifth days.

The dog took some food each day, but on several days did not finish all its food.

TABLE XI

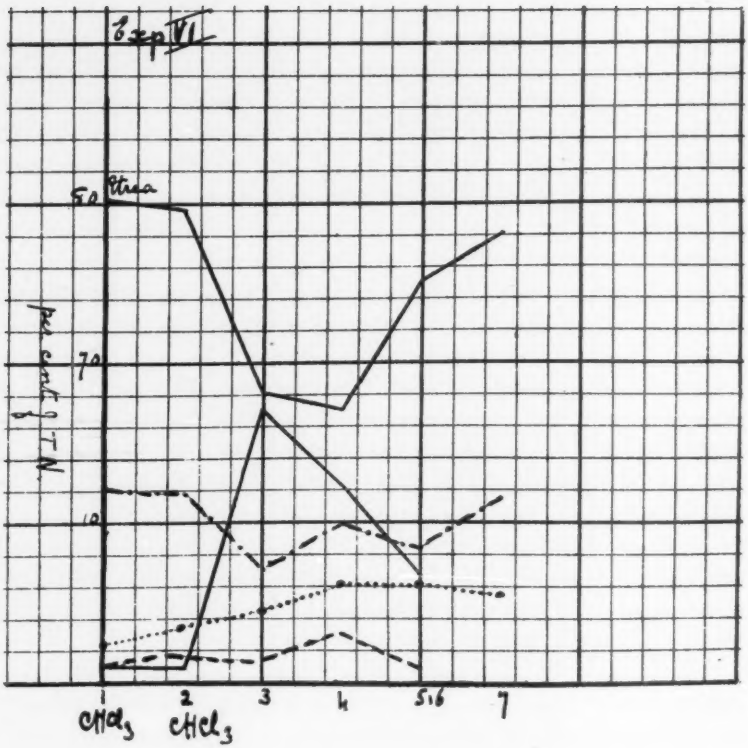
Day	Total N	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	7·7	0·194	6·197	0·077	0·066	0·179	0·051	0·936
2*	6·475	0·229	5·143	0·060	0·119	0·167	0	0·759
3	10·087	0·446	6·869	0·740	0·162	0·188	0·007	0·675
4	7·91	0·498	5·312	0·924	0·232	0·179	0	0·765
5 & 6	6·581	0·412	4·958	0·416	0·060	0·149	0·018	0·566
7	5·6	0·316	4·374	0·266	—	0·146	0·025	0·644

TABLE XII

PERCENTAGE OF TOTAL NITROGEN

Day	Ammon. N	Urea N	Allantoin N	Amino acid N	Creatinin N	Creatin N	Und. N
1*	2·5	80·5	1·0	0·9	2·3	0·6	12·2
2*	3·5	79·4	0·9	1·8	2·5	0	11·9
3	4·4	68·1	17·2	1·6	1·8	0·1	6·8
4	6·2	†67·2	11·7	2·8	2·2	0	9·9
5 & 6	6·2	75·3	6·3	0·9	2·2	0·3	8·6
7	5·6	78·1	—	—	2·6	0·4	11·6

EXPERIMENT VI



General Conclusions

The results bear out those obtained by Professor D. Noël Paton.

Total nitrogen.—In every case there is a distinct increase in the total nitrogen excreted, an increase which is especially well marked in Experiments II, V, and VI.

Urea nitrogen.—In each case there is a distinct fall in the proportion of urea nitrogen, reaching its maximum on the third day after the administration of chloroform. In Experiment III a slight rise in the proportion of urea precedes the fall, and the maximum decrease is, in this case, not reached till the fifth day after the drug was administered.

Ammonia nitrogen.—The proportion of nitrogen in the form of ammonia rises, reaching its maximum as a rule on the fifth day after the administration of chloroform.

Allantoin nitrogen.—In Experiments I and II, in both of which the chloroform was administered by the mouth, the proportion of allantoin nitrogen shows a gradual but distinct increase, which in II does not reach its maximum till the sixth day after the administration of the chloroform. In the other experiments in which the drug was given hypodermically, the maximum increase is reached on the second day after (the fourth day in III), and is followed immediately by a sharp fall.

The significance of allantoin in the urine of the dog is not fully understood. Many observers consider that it is the end product of purin metabolism, while others are inclined to look upon it as an intermediate product which may undergo further change.

Wiechowski, for example, holds that it is an end product, and considers that the appearance of allantoin in the dog's urine is caused by the splitting of the uric acid. In man this decomposition occurs only to a slight extent, hence the replacement of allantoin by uric acid in the human urine. Sucklings, he states, seem to occupy an intermediate position with regard to this power of decomposing uric acid, and allantoin is found in their urines in considerable amounts. His observation that in the dog it is possible to recover completely the allantoin after injection confirms this view.

The fact that Pohl[1] found allantoin in the tissues of a dog poisoned with hydrazine, and Underhill and Kleiner[2] in the urine, but not in the normal animal, and the observation of Borissow[3] that allantoin appears in the urine after diamido sulphate poisoning, seem to indicate either that it is an intermediate product, or that these poisons, by liberating nucleins, yield a larger supply of purins to be converted into allantoin. The increase of allantoin in the present experiments might be explained on either hypothesis.

Amino acid nitrogen.—The administration of chloroform causes in each case a rise in the proportion of amino acid nitrogen. In I and IV, the experiments on the collie dog, the rise is very marked; in II and III, on the contrary, those on the brown retriever, the increase corresponds rather to an appearance of amino acids, as in the normal urine of the brown retriever only a trace of amino acids could be found and on several days of the experiment they were completely absent.

The peculiar reaction of this dog is of considerable interest. Instead of the usual rise in mon-amino acids the undetermined nitrogen rose. This led to the consideration of the possibility of this being due to the appearance of products of the nature of diamino acids and polypeptides.

In Experiments II and III, therefore, where the amount of mon-amino acid nitrogen is small an attempt was made to ascertain approximately the amount of nitrogen present in the form of diamino acids.

The phosphotungstic acid precipitate was decomposed with barium hydrate and barium carbonate, the excess of barium removed with CO_2, and silver nitrate added. The nitrogen in the silver precipitate was estimated by Kjeldahl's method. It probably consists to a very large extent of the silver salts of the diamino acids.

	Und. Nitrogen	Ag prec. Nitrogen
Experiment II	12·7	4·9
	10·1	7·6
Experiment III ...	19·0	18·5

It would thus seem as if, in this animal, diamino acids rather than monamino acids formed intermediate products of catabolism. Gideon

1. *Archiv. f. exp. Pathol.*, XLVI, p. 367.
2. *Journ. of Biol. Chem.*, Vol. IV.
3. *Ztsch. f. phys. Chem.*, XIX.

Wells[1] found that in cases of delayed chloroform poisoning very considerable amounts of diamino acids could be isolated from the abnormal livers. Diamino acids also are said to be excreted in cases of cystinuria.[2]

Creatinine nitrogen.—There is no very marked change in the amount of creatinine nitrogen. The proportion appears to fall slightly on the day after the administration. This agrees with the work of Howland and Richards,[3] who found creatinine remained fairly constant and consequently that there was a percentage decrease.

Creatine nitrogen.—The effect of chloroform is to cause the appearance of creatine in the urine two or three days after its administration at the time when the general disturbance in the metabolism is most marked. Howland and Richards observed a marked increase in the percentage of creatine in the two cases of delayed chloroform poisoning.

In Experiments III, IV, VI, the dog took its food each day, and the appearance of creatine which occurs in these may be considered as an effect of the chloroform. In Experiments I, II, V, in which food was refused for some days after the chloroform, the fact that starvation is a cause of the appearance of creatine must be taken into account. But in Experiment V the appearance of creatine takes place after the animal had resumed its food, when the starvation effect may fairly be held to be ended.

This disturbance in the creatine metabolism is almost certainly to be associated with the disturbance in the hepatic functions, since Mellanby[4] found creatine was excreted in great amount in carcinoma of the liver; Underhill and Kleiner[5] observed fatty degeneration of the liver and creatine excretion following injection of hydrazine sulphate; and Hoogenhuyze and Verploegh[6] state that the amount of creatine present in the urine may be taken as an indication of the extent of the hepatic disturbance.

Undetermined nitrogen.—The proportion of undetermined nitrogen shows in each case a rise of from 5 to 9 per cent. Normally about 4 to 5

1. *Journ. of Biol. Chem.*, Vol. V, 1908-9.
2. Loewy u. Neuberg, *Ztsch. f. phys. Chem.*, XLIII.
3. *Loc. cit.*
4. *Journ. of Physiol.*, Vol. XXXVI.
5. *Loc. cit.*
6. *Ztsch. f. phys. Chem.*, LVII, p. 264.

per cent. of the total nitrogen is undetermined. The greatest increase occurs on the same day or the day after the maximum fall in the proportion of urea nitrogen, the rise in the proportion of nitrogen in the form of ammonia, allantoin and amino acids not being sufficient to counter-balance this decrease.

This fact, and the consequent increase in the undetermined nitrogen, leads to the question: To what is this undetermined nitrogen due? It occurs altogether in the phosphotungstic acid precipitate. Creatinine, which is also included, is separately estimated, the amount of purin nitrogen is quite negligible, so that in all probability this undetermined nitrogen consists of diamino acids and polypeptides.

With regard to the possible presence of polypeptides in the urine the formol titration method seemed to offer a convenient and speedy means for the estimation of the nitrogen in this form.

Henriques and Sorensen[1] have evolved a method whereby the polypeptides are decomposed by heating with hydrochloric acid, and the amino acids thus split off are estimated by formol titration. They by this method found polypeptides to be normally present in small amounts in the urine of dogs—0·0 to 1·6 per cent. of the total nitrogen.

The following table shows the results obtained which prove the presence of lower proteins and polypeptides in considerable amounts. The higher proteins present were first removed by precipitating with trichloracetic acid:—

Day	Und. Nit. Grs. nit.	Polyp. Nit. Grs. nit.	Und. Nit. % T.N.	Polyp. Nit. % T.N.	Still Und. % T.N.
Experiment V:—					
3	2·11	2·23	13·0	13·6	– 0·6
4	0·822	0·451	10·8	5·8	5·0
5	0·332	0·397	6·3	8·9	– 2·6
6	0·327	0·259	5·2	4·9	0·3
7	1·269	1·035	13·3	12·0	1·3
Experiment VI:—					
1	0·936	0·819	12·2	10·5	1·7
2	0·759	0·406	11·9	6·3	5·6
3	0·675	0·261	6·8	2·5	4·3
4	0·765	0·467	9·9	6·0	3·9
5	0·473	0·427	9·0	8·0	1·0
6	0·660	0·362	8·6	4·5	4·1

The disturbance in the nitrogenous metabolism revealed by these experiments appears to be primarily due to the disturbance in the

1. *Ztsch. f. phys. Chem.*, LXIII and LXIV.

function of the liver which is so clearly indicated by the structural changes described by Clark.[1]

It has been maintained that they are due to an acidosis produced by the administration of chloroform. That such an acidosis occurs in the human subject there can be no doubt, but in the dog this is not manifest, although the changes in the distribution of nitrogen are quite marked. Underhill and Kleiner[2] got no evidence of the presence of acetone oxybutyric acid, etc., and Richards and Wallace[3] found acetone was not present after cyanide poisoning. I myself also failed to find any indication of an increase of acetone and diacetic acid in the urine following on the administration of chloroform. Further work is at present being done on this question.

Hence it seems that the acidosis, when it occurs, is, like the disturbance in the distribution of nitrogen, secondary to the interference with the hepatic functions.

I take this opportunity of acknowledging my deep indebtedness to Professor Noël Paton, under whose guidance this work has been done, for carrying out the experimental work and for much help and criticism.

1. *Loc. cit.*
2. *Loc. cit.*
3. *Journ. of Biol. Chem.*, Vol. IV.

THE ACTION OF CERTAIN SULPHUR COMPOUNDS ON METABOLISM AND EXCRETION

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The Sulphates. There has recently been much dispute as to whether the soluble sulphates exhibit their well-known purgative effects when administered otherwise than by the alimentary tract. In the present experiments it has been shown that no purgative action follows administration of sodium sulphate hypodermically; but there is, however, a slight increase in the quantity of the faeces, this increase not commencing until twenty-four hours after administration and terminating about twelve hours later. These faeces contain sulphates in much larger amounts than at other times. Several noteworthy metabolic changes were also noticed as the result of hypodermic injection of sulphates.

The animals used in all experiments were rabbits. The food was carefully weighed daily. The urine, and in a few instances the faeces also, were collected and subjected to examination and estimation of the various constituents. Sulphates were estimated by Folin's method. Five c.c. of the urine was used in each instance, and after acidulation with dilute hydrochloric acid, barium chloride solution was added drop by drop. After standing all night the precipitate was filtered off, ignited and weighed.

Five c.c. of the urine was heated with two per cent. hydrochloric acid for an hour, and to it barium chloride solution was added drop by drop; the precipitate after standing two hours was filtered, ignited and weighed. The difference between these two determinations gave the amount of sulphates combined with aromatic substances. Urea was estimated by Little's nitrometer; nitrogen by Kjeldahl's method; the other constituents in the urine were estimated by the usual methods.

It is interesting to note that the hypodermic injection of sodium sulphate does not affect the sum total of metabolic range to any great extent. Following small doses (up to one gram) the animal continued to gain weight, very much as before, even after somewhat prolonged administration. Large doses seemed to lessen the gain, but even these animals continued to gain weight slowly.

Very small doses seemed to cause a slight increase of the moisture, and to a less extent of the quantity of the faeces, but as the dose increased the faeces became less in quantity and contained less moisture.

A small hypodermic injection (0·5 gram) was followed by a very marked increase in the volume of urine excreted; this amounted to about three times the normal volume. Each constituent of the urine was markedly increased to about three times the normal amount, and in the case of the inorganic sulphates there was usually a still greater increase.

The following day the volume of urine and amount of each of its constituents is slightly diminished, and in from five to six days the urine has returned to its normal condition.

It is remarkable that the weight of sulphates excreted during these six days is much greater than the sum of the amount contained in the food and the amount given in the hypodermic injection.

Urinary volume in c.c.	Urea	Inorganic sulphates	Aromatic sulphates	Chlorides	Phosphates	Ammonia	Uric acid	Nitrogen in other forms
150	0·486	0·506	0·045	0·0942	0·0410	0·0597	0·0782	0·0161
154	1·709	2·156	0·154	0·3719	—	0·2042	0·2232	0·170
138	1·366	1·476	—	0·4540	0·0927	0·0844	0·0579	—
125	0·825	0·925	0·1625	0·2131	—	0·0642	—	—
50	0·351	0·780	0·0225	0·1616	0·0432	0·0399	—	—

As the amount of sulphate is increased, the marked diuresis disappears; the amount of sulphate necessary is about two grams, but varies considerably. The most noteworthy feature of the urine during the twenty-four hours following a large injection is the increased excretion of inorganic sulphate accompanied by a diminished excretion of aromatic sulphate; this becomes more marked as the amount of sulphate is increased. The volume of urine and weight of its other constituents are not altered during this first twenty-four hours. The ratio of aromatic sulphate to inorganic diminishes from one to ten till it becomes one to sixty-two, the aromatic sulphates being one-twentieth their normal quantity. The amount of sulphate excreted in the faeces during the same twenty-four hours was about normal, being one-fifth that excreted in the urine.

The following day the diuretic action took place; the volume of urine was increased over three times the normal amount. Large quantities of inorganic sulphates were got rid of, and the aromatic sulphates were greatly increased in amount to about eight times the normal amount. The ratio of organic to inorganic sulphates was now

one to four. Urea was not as large as it should be in proportion to the amount of the other constituents, but these were increased in proportion to the increase in urinary volume.

Irritation of the renal cells was evidently present, there being casts in the urine, both epithelial, and more especially granular. These casts were first noted after one gram of sulphate had been given, so that it probably has a very irritating action on the renal cells, and probably on other living cells. Insoluble phosphates of calcium and magnesium were invariably present about forty-eight hours after an injection of sulphates; with larger doses calcium carbonate and calcium oxalate were also found.

Urinary volume in c.c.	Urea	Inorganic sulphates	Organic sulphates	Chlorides	Phosphates	Ammonia	Uric Acid
50	0·486	0·506	0·045	0·0942	0·0410	0·0597	0·0782
63	0·605	1·121	0·0063	0·1541	0·0759	0·0525	0·0822
162	0·907	1·247	0·3402	—	—	0·1157	0·0656
130	1·728	1·092	0·2080	—	—	0·0322	0·0692

During the first twenty-four hours the excretion of sulphates in the faeces was about normal; during the next twenty-four hours corresponding to the diuretic action shown in the above table there was a sudden increase in the amount of sulphate excreted in the faeces. The amount excreted in this way was three times the normal amount, and corresponded roughly to the increase in the urine. The amount normally excreted in the faeces is about one-fifth of the amount given out in the urine. After the hypodermic injection the faecal excretion of sulphate became seven-ninths of the urinary excretion. The following day the excretion of sulphates fell again to normal, and continued so.

Larger doses hypodermically caused exaggeration of these symptoms; two or more days now supervened before the diuretic effects were seen. The aromatic sulphate excretion became exceedingly small, being one two-hundredth the amount of the inorganic. A certain quantity of sulphate seemed to be required to be excreted before the rise in metabolism occurred, and the greater the quantity injected the longer time interval was required before the concentration of sulphates in the blood had been brought down to such a point that a metabolic rise occurred and the animal commenced to return to its normal condition.

With the large doses, casts almost entirely granular were always found in the urine in large quantities; such a marked irritation of the renal cells would point to a very irritating action by the sulphates.

Phosphates of calcium and magnesium were present after each injection. With small doses of sulphates, carbonate and oxalate of calcium were present, but these two salts increased very greatly in amount as the dose of sulphate was increased.

Experiments in Autolysis. An attempt was made by the aid of autolysis to find out if sodium sulphate had any effect on the metabolic changes in living cells. For this purpose 10 grams of finely-minced fresh liver were used in each experiment, using every aseptic precaution; to this minced liver was added fifty cubic centimetres of sterile distilled water, two cubic centimetres of toluol, and various quantities of sodium sulphate. A control without any sulphate was also made. All the preparations were placed in an incubator at thirty-eight degrees Centigrade for three days. Each was then boiled, filtered, and the filtrate and solid portion were then estimated separately for total nitrogen by Kjeldahl's method.

It was found that there was slightly more autolysis in the control, but not more than was found to be present when any other salt was used instead of the sulphate.

From these results it would seem that sodium sulphate in small amounts has a stimulating effect on the body cells, especially those of the renal and, to a less extent, of the intestinal epithelium. There is, however, a point above which this stimulant action becomes less marked, this being probably due to the concentration of sulphates in the blood being too high. This high concentration limits to a more or less extent, according to the amount of sulphate present, the normal interchange of products between the blood stream and the individual body cells. This is shown by, firstly, the greatly diminished excretion of aromatic sulphates from the cells, and the absence of the strong metabolic stimulus shown by the marked diuresis which follows small doses.

During this first twenty-four hours after a large dose, sulphate, almost entirely as inorganic sulphate, is being excreted, and when the blood concentration of sulphate is reduced to a certain point there is an immediate metabolic stimulus and the marked diuretic effect is seen, and at the same time a greatly increased amount of aromatic sulphate is thrown out, showing definitely that the sulphate has not had a poisonous effect on the cells, but rather has produced a barrier between the blood and the living tissue cells. The action of sodium sulphate hypodermically on the intestine is very similar but much less marked, for when the sulphate is in high concentration in the blood there seems to be little

or no power to get rid of it through the intestinal epithelium; but when the kidney has reduced the concentration to the necessary point, there is then a large increase in the sulphate excreted through the intestinal tract. It must, however, be pointed out that the weight of sulphate excreted by the intestinal tract amounts only to about 0·8 gram, and as the kidney is excreting more than three times its normal volume of water during this day, it is hardly strange that no marked purgative action follows *hypodermic* injection of sodium sulphate, and, as mentioned above, more sulphate injected into the blood represents more work for the renal epithelium to do before the point is reached when the marked diuresis occurs. An increased amount of sulphate is simultaneously given out in the faeces, and this increased intestinal excretion only lasts for a few hours, the excretion next day by that route being normal.

The marked irritation of the renal epithelium as shown by the presence of casts, both epithelial and granular, in the urine after the hypodermic injection of sodium sulphate, is important. This was first noticed when less than one gram had been injected. As the dose was increased, casts became more and more abundant. As malted drinks contain large quantities of sulphates, and as those addicted to such drinks seem especially prone to such diseases as chronic renal disease and cirrhosis of the liver, both diseases probably due to some toxic irritation, it seems possible that the sulphates present may be at least one cause of such conditions, and not the alcohol alone, which is not present in very large quantities. Lastly, the presence of calcium phosphate, carbonate and oxalate found in the urine after injection of sulphates, and probably due to some interference with metabolism, is curious in that these salts are very often present in the urine of drinkers of malted liquor.

The Sulphites. The metabolic action of sodium sulphite administered hypodermically in moderate doses on animals is as yet unknown. The effect of a poisonous dose is to kill by depressing the heart and circulation generally, and also the respiration. The same methods were adopted as in the case of the sulphates, the solution of the drug, freshly prepared, being injected hypodermically in all cases.

Hyposulphite of sodium was first used. It was found that after hypodermic injection there followed a marked diuresis, the urinary volume being about three times the normal amount and all the urinary constituents being increased. Casts, both epithelial and granular, were present, and more marked than after similar doses of sulphate, and there

followed even larger quantities of phosphates, carbonate and oxalate of calcium, than after sulphate injection.

It will be seen, therefore, that sodium hyposulphite acts very similarly to sodium sulphate, and is so rapidly oxidised in contact with the body cells that the effects may be considered as due to sulphates.

Sulphite of sodium was next investigated. The following day, after the hypodermic injection of 0·1 gram, there was an increase in the urinary volume, and also in the urinary constituents, an effect resembling that due to sulphates, and probably due to a portion of the sulphite being oxidised to sulphate. With this action there was found a large increase in the normal colouring matter in the urine, the presence of granular casts, and also of insoluble calcium salts. These effects are, however, not found after a dose of sulphate corresponding to that which the whole of the injected sulphite would yield on oxidation, so they must, in part at least, be accredited to unoxidised sulphite action in the blood. The following day the urine was diminished; there was still excess of colouring matter, and insoluble calcium salts, mostly phosphatic.

On the third day the volume of urine was greatly increased, a large amount of sulphates were thrown out, together with a great excess of calcium and magnesium salts—chiefly carbonate with some phosphates and oxalates—and a large number of granular casts were present.

Amount	Urea	Inorganic sulphate	Organic sulphate	Chlorides	Phosphate	Ammonia	Uric acid
45	0·398	0·386	0·048	0·1246	0·0211	0·0407	0·0510
90	1·368	0·426	0·054	0·1820	0·0810	0·0474	0·0623
24	0·392	0·3321	0·031	0·1210	0·0341	0·0418	0·1011
140	1·706	1·218	0·162	0·2600	0·0711	0·879	0·0631

As the dose of sodium sulphite is increased the stimulant action of the sulphate produced from it by oxidation is more and more obscured, and the depressent action of the unaltered sulphite becomes more marked. Thus, after injection of half a gram of the sulphite hypodermically, during the following three days only thirty cubic centimetres of urine were passed. This amount is much less than is normally passed in one day. There was, moreover, no increase in the amount of sulphates thrown out, showing that the sulphite had an inhibiting action on the oxidation processes which would change it into sulphate. At the same time, a great excess of the ordinary urinary pigment was present, together with a very large quantity of insoluble salts, so that the urine was milky in

colour and consistency. This salt was found to be almost entirely calcium carbonate, with a smaller quantity of calcium phosphate and oxalate, accompanied by many granular casts. On the fourth day sulphates commenced to be excreted above the normal amount, the top level of sulphate excretion with this amount of dose being attained on the sixth day after injection.

A still larger hypodermic injection was then given, amounting to one gram of the sulphite. No urine at all was passed during the following two days. The following day a quantity just above normal, of a thick, cloudy, dark orange-coloured urine, was passed. This orange colour was found to be due to the normal urinary pigment in excess. All the constituents of the urine, including sulphates, were about normal. Casts were present in large quantities. The thickness and cloudiness of the urine was due to carbonate of calcium, with some phosphate and oxalate, and also traces of magnesium salts.

The following day the animal cells had begun to overcome the depressing action of the sulphite, for although the urine was not increased in amount the urea began to rise in amount, as also the sulphates. The following day there was no urine again, and this was next followed by a rapid rise in the amount of urea formed and the amount of sulphate excreted, showing that the sulphite had been oxidised and excreted.

Amount	Urea	Inorganic sulphate	Organic sulphate	Ammonia	Uric acid
Nil	—	—	—	—	—
Nil	—	—	—	—	—
60	0·408	0·305	0·012	0·0163	0·0288
63	0·931	0·882	0·246	0·0083	—
Nil	—	—	—	—	—
87	0·731	1·566	0·139	—	—
160	1·088	1·104	0·112	0·0487	0·0127

In these hypodermic injections of sodium sulphite the enormous excretion of calcium carbonate, and to a less extent calcium phosphate and oxalate, was a most noticeable feature; this deposit, after settling, was about half the bulk of the whole urinary volume. The quantitative estimation of these salts will be found in the section on sodium sulphide, which was also found to produce them in large amount.

It was found that different animals differed very much in their reaction to sodium sulphite; in other words, they differed in their oxidising powers. The previous rabbit had reacted strongly to one gram

of sodium sulphite. The same quantity hypodermically injected into a fresh rabbit produced death in half an hour, with the usual well-known symptoms, viz., depression of the circulation and respiration; while a third rabbit oxidised half a gram of sulphite so quickly that the symptoms were similar to sulphate injection. Even one gram in this animal produced only similar symptoms to that shown by the first rabbit after one-tenth of a gram.

Experiments in Autolysis. The effects of hyposulphites and sulphites of sodium were then tried on autolysis. Ten grams of finely minced liver were added to fifty cubic centimetres of sterile distilled water, using every aseptic precaution. Two cubic centimetres of toluol were added. This served as a control. To similar preparations various amounts of hyposulphite and sulphite were added, and the whole placed in an incubator at thirty-eight degrees Centigrade for seventy-two hours. Each was then boiled, filtered, and total nitrogen in the liquid and solid portions each separately estimated by Kjeldahl's method.

It was found that hyposulphite had no appreciable effect. Sulphite, however, was found to be different; up to a certain amount it had no effect on the rate of autolysis, but when that point was passed there was an almost immediate stoppage of all autolysis.

	Nitrogen in soluble part	Nitrogen in insoluble part
Normal liver, boiled before autolysis commenced	0·0381	0·2836
Normal liver autolysis	0·1671	0·1532
Liver with sulphite	0·0428	0·2762

It would therefore appear that sulphite has an inhibiting effect on autolysis, due probably to combination with the enzymes of the cell. But as the effect of sulphite on an animal is very variable, depending on the oxidation powers of that animal, so that the effect of sulphite on autolysis was found to be very different in different tissues. In some liver tissue, ten grams always being used, as small a quantity as two decigrams was sufficient to arrest all autolysis, while in another liver five decigrams were necessary, and in some cases very much larger quantities were required; but in all cases there is a fixed point which may be called the oxidation limit, any sulphite above which being added will fix the ferments and cause the stoppage of all metabolic changes.

From these experiments it appears that when the sulphite is too great for the oxidation powers of the cells, it immediately combines with the ferments and in this way stops all metabolic tissue changes, and

death results. But this result does not show us whether the sulphite becomes attached to the cell and is there attacked, or whether the oxidising ferments attach themselves to the sulphite, so that it is only when the sulphite is in excess that it becomes attached to the cell.

A rat was given a large dose of sulphite of sodium hypodermically, and a few minutes afterwards it was killed, as well as another untreated rat to serve as a control. On submitting the livers to autolysis for twenty-four hours no difference was found in the rate of autolysis; the sulphite liver, in fact, had progressed further than the control, which points to the fact that the sulphite becomes anchored in the body to oxidising ferments.

As a further test, a large dose of sulphite (one gram) was given to a rat, and an hour and a half afterwards the rat was killed, its liver, spleen and kidneys removed, finely minced, and placed in a small flask connected with glass tubing to another small flask containing a weak solution of barium chloride. Two per cent. hydrochloric acid was added to the tissues, which were then heated. Not the slightest trace of barium sulphite was produced, nor in substituting a weak solution of copper sulphate was any insoluble copper sulphite produced, which tends to show that the sulphite does not long exist as such in the living cells.

From these results it appears that sodium sulphite exerts two distinct actions in the animal body. One action is due to sodium sulphate into which part of it is almost immediately oxidised, and the other action is due to excess of free sulphite which the body is unable to cope with. The proportion of these two depend in the first place on the dosage; a small dose acting almost entirely as sulphate, a large dose obscuring the sulphate action by the more energetic action of the sulphite. Animals differ very considerably in their oxidising powers; one will show sulphite action after two decigrams of sulphite, while another and apparently similar animal, will show very little sulphite action after one gram. The sulphate action consists of an intense metabolic stimulation, increased urine volume, urea, and other constituents. The sulphite action is a depressent action attacking the cell ferments and inhibiting them; this is shown by its action on metabolism, and also by its powerful inhibiting action on autolysis.

This action on the cell ferments appears to be somewhat complicated. The sulphite seems to combine with some part of the ferment, but apparently does not destroy it, for no compound containing sulphite can be found in the cells, while at the same time the cell ferments are

completely inhibited. If the sulphite is only sufficient to neutralise part of the ferment the animal recovers, oxidising ferments are brought in contact with the cells, and the sulphite is oxidised gradually to sulphate. Such an occurrence has been often observed in animals which have been apparently dead with sulphite poisoning, recovering completely.

The complete inhibition of the tissue ferments produces general symptoms usually described as central; that is, there is failure of respiration and also of circulatory centre, together with convulsions.

The sub-lethal action is as follows: There is produced an intense irritation of the renal epithelium which becomes more intense as the dose is increased; this is shown by the excretion of large quantities of casts, both epithelial and granular. There is also an interference with oxidation processes in the cells; this is shown by the presence of excess of the normal colouring matter of the urine and the presence of large quantities of calcium, and to a less extent magnesium salts, in the urine. These calcium salts are in the form of phosphate, carbonate and oxalate.

Sulphide. The sulphides are credited with having some action on suppurative processes; it was therefore sought to find out if there were any grounds for such an action.

As in the case of sulphates, hyposulphites and sulphites, all doses were given hypodermically. Great difficulty, however, was experienced owing to the extremely caustic nature of sodium sulphide. Kahlbaum's sodium sulphide was used for the first experiments, but Dr. Alexander Rule, Chemical Department, University of Liverpool, was kind enough to supply me with a quantity of sodium hydrogen sulphide of definite composition, the manufacture of which he had succeeded in discovering by precipitation in alcoholic solution. This preparation was found in every way more satisfactory, especially in its diminished causticity.

The first dose given was two centigrams of Kahlbaum's sodium sulphide, hypodermically. There was no urine passed during the following twenty-four hours, and this was followed next day by a small quantity of urine containing a diminished quantity of all the urinary constituents with the exception of sulphate. Casts were found in the urine, and also large quantities of insoluble calcium and magnesium salts, chiefly calcium carbonate and phosphate, with some calcium oxalate.

Injection of four centigrams of the sulphide was followed by two days' anuria; there was the following day a slight increase in the volume of urine and amount of urea and inorganic sulphates, but a

diminished excretion of organic sulphate. The insoluble calcium salts were present. Organic and inorganic sulphate was on this day as one is to thirty. The following day there was a large quantity of a substance which, when the urine was boiled with weak hydrochloric acid, smelled of creosote, and was probably combined with sulphate. The organic sulphate on this day had risen to one to five of inorganic. In all the following experiments the new sodium hydrogen sulphide of Rule was used.

Three decigrams of sodium hydrogen sulphide (0·3 gram) were given hypodermically to a rabbit. Within a short time its respirations became laboured and feeble; at the same time its heart beat became weak, and within three minutes it had a convulsion and died.

A fresh rabbit was given, hypodermically, five centigrams (0·05 gram). No urine was passed the following twenty-four hours; the following day, although the urine was less in quantity than normal, there was an increase in the urea and uric acid excreted; all the other constituents, including inorganic sulphates, were decreased; organic sulphates were slightly increased.

Amount	Urea	Inorganic sulphate	Organic sulphate	Ammonia	Uric acid
45	0·468	0·4500	0·045	0·0355	0·0310
Injection 0·05 gram sodium sulphide.					
No urine	—	—	—	—	—
40	0·900	0·166	0·048	0·0082	0·1216
42	0·496	0·290	0·068	0·0357	0·0464

A very large amount of insoluble salts were excreted on the third day. The sediment of these in bulk amounted to about one-third the urine volume. On shaking up, the consistency was extremely thick. These salts consisted chiefly of calcium carbonate, phosphates, chiefly calcium, and lesser quantity of calcium oxalate; there were also small quantities of magnesium salts. The carbonates in this urine were estimated, and represented four decigrams of calcium carbonate; there was nearly a gram of insoluble salts in this urine. Casts also were present in large quantity. A second rabbit reacted strongly to sodium sulphide, resembling the one previously given Kahlbaum's sulphide. One decigram of sulphide produced two days' anuria.

Another rabbit was given six centigrams of sodium sulphide hypodermically. There was an immediate diuresis closely resembling that seen after a dose of sulphate. Urine-volume, urea and other

constituents were roughly three times the normal amount. Total sulphates were very much increased, as also were organic sulphates. The sulphates were increased out of all proportion to the amount of sulphide given; nearly a gram and a half were excreted during the twenty-four hours. Casts, carbonates, phosphates and oxalates, chiefly of calcium, were present. A larger dose in the same rabbit of one decigram, hypodermically, gave a combination of the two effects. Normal amount of urine and its constituents, except slight increase in excretion of urea and excretion of insoluble calcium salts and casts, followed next day by the diuresis resembling sulphate diuresis. So that sulphide appears to resemble sulphite in its action in that it depends on the oxidation powers of the animal as to whether the injected compound should give the stimulating effects of sulphate, or the depressing effects of sulphide.

Experiments in Autolysis. These were performed in similar manner to those attempted in regard to sulphates and sulphites. Ten grams of fresh liver tissue was used in each case, toluol being used as a preservative. Very small quantities of sodium hydrogen sulphide were added. It was found, after incubating for seventy-two hours, that practically no autolysis had taken place in the ones containing sulphide, but, as with sulphites, tissues were found to differ very much in their resistance to this substance.

Lastly, it appeared of interest to find out whether sulphide formed a compound with any part of the living cells or whether it only became attached to the ferments. A rat was given two and a half decigrams of sodium hydrogen sulphide, hypodermically, and about half an hour afterwards was killed; the liver, spleen and kidneys were quickly removed, minced, ground in a mortar, and placed in a flask connected with another flask with glass tubing, the second flask containing a little copper sulphate. Two per cent. hydrochloric acid was added to the tissues, which were then heated. A distinct production of copper sulphide was discernible where the tube dipped into the copper sulphate, which would tend to show that sulphide forms a compound with some substance in the cells. A control rat was used, the organs in this case giving no sign of any sulphide.

We now attempted to further investigate the production of insoluble salts after an hypodermic injection of sodium hydrogen sulphide. One decigram was given hypodermically; the following day there was complete anuria.

The second day there was thirteen cubic centimetres of a dark urine, containing casts. The phosphates were the most prominent insoluble salts, somewhat less oxalates, and only a trace of carbonates.

The third day forty centimetres were passed. Phosphates were still in excess, carbonates had increased, oxalates decreased.

The fourth day thirty-five cubic centimetres; the oxalates had further decreased, carbonates increased, triple-phosphates increased.

The fifth day one hundred cubic centimetres of urine, sufficient to estimate the substances quantitatively. There was found to be present: calcium carbonate, 0·25 gram; calcium oxalate, 0·007 gram; calcium phosphate, 0·044 gram; ammonium and magnesium phosphates, 0·160 gram. The following day the urine was clear.

From these results it would appear that sodium sulphide, hypodermically, may have two separate actions. Firstly, in some animals possessing large quantities of oxidising ferments the sulphide may be almost immediately oxidised to sulphate, and then the action will be the pronounced diuretic action seen after injecting sulphate. Other animals having less oxidising ferments in their tissues will cause little or no oxidation of the sulphide, and the effects produced will then be the depressing effects due to the sulphide. Again, other animals will be found to be intermediate between these two, and there will be seen the mixed effect of the depressing sulphide and the stimulating sulphate.

If the dose of sulphide is lethal it combines with the cell tissues, or possibly the cell ferments, and produces cessation of all metabolic changes. The signs of such an action are paralysis of the respiratory and circulatory systems, convulsions, and death.

If the dose is sub-lethal, oxidase and other oxidising ferments attack the sulphide, which is then thrown off the cell in the form of sulphate, and this production of sulphate under such conditions is frequently in such minute amounts that no diuresis such as is seen after sulphate is to be found.

This sulphide combination in the cells produces a depressent action on tissue changes, but more especially on oxidation, so that unfinished products of oxidation are excreted. Calcium phosphate, carbonate and oxalate are invariably present in the urine, the carbonate being the most unusual and prominent, although the phosphates are found in larger amounts. In one day's urine four decigrams of calcium carbonate were found, and there were, besides, large quantities of phosphates and other salts. In another urine, on the fifth day after injection, two and a half

decigrams of calcium carbonate were found, together with about two decigrams of other insoluble salts.

Lastly, sulphides exert an extremely irritating effect on the renal epithelium, shown by the presence of casts in the urine after each hypodermic injection of the substance.

Conclusions

Sulphates, hyposulphites, sulphites and sulphides interfere with oxidation processes in the cells, sulphates in large amount by preventing exchange between the blood stream and the cells. This action disappears when the amount of sulphate is reduced, and there then follows a stage of stimulation, the sulphate causing a very marked diuresis, sometimes accompanied by the excretion of much more sulphates than were injected into the body together with the amount found in the food. Sulphate also has a marked irritant action on the kidney cells when more than a small amount is present. Purgation is not usual; during the second day after an injection an increased quantity of sulphates is excreted in the faeces, but the following day the amount has again fallen to normal. Calcium carbonate, phosphate and oxalate are found in the urine after large doses, and are probably products of incomplete oxidation, the oxidation having been interfered with by the high concentration in the blood preventing interchange with the cells.

Hyposulphites act very much like sulphates, being very quickly reduced in the body to sulphate.

Sulphites and sulphides are somewhat similar in their action, and this action depends on the amount of oxidising ferments found in the animal. In some animals they are at once oxidised, and the sulphates produced give the typical picture described above. If the animal is deficient in oxidising ferments the sulphite appears to unite with and neutralise the action of the tissue ferments. Sulphide appears to unite to some constituent of the cell itself, and both interfere, according to the quantity, with tissue changes, and if in sufficient amount they stop all metabolic changes and the animal dies of failure of respiration and circulation, with perhaps convulsions. If the amount is sub-lethal, it is attacked by the oxidising ferments, and gradually got rid of as sulphate. In the case of sulphite, when the toxic dose is large the sulphate gives the diuretic action, but in the case of sulphide the amount of sulphate is so minute that little or no diuresis is often to be seen.

Both sulphite and sulphide interfere with metabolic changes, especially with oxidation processes, causing the excretion of calcium especially, but also of other insoluble salts in the form of carbonate, phosphate and oxalate. There is also an intense renal irritation as the result of both these salts. The irritation caused by all these three sulphur compounds raises the possibility as to whether they may not, in part at least, be the cause of chronic nephritis and cirrhosis of the liver, both frequently ascribed to malted drinks, which are known to contain large amounts of sulphates. When it is also considered that these people usually have insoluble salts in their urine, such as are described in this paper, and as such results are not common after drinks which are free from sulphates, it is possible that the sulphates, and not the alcohol, may be the cause of their condition.

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URIC ACID METABOLISM IN RABBITS

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In previous experiments[1] an attempt was made, by perfusing the surviving liver of the dog with sodium urate solutions, to ascertain whether allantoin was the sole end product of uric acid metabolism, or whether some fraction went by another line of decomposition to urea. The results obtained were inconclusive, for although no urea could be isolated, the amount of uric acid which disappeared was considerably greater than could be accounted for by the quantity of allantoin obtained, and it was shown that this was not due to a further destruction of allantoin.

Bezzola, Izar and Preti[2] have shown that, when diluted defibrinated blood containing uric acid is perfused through a dog's liver with a plentiful supply of oxygen, a considerable amount of the uric acid disappears, but that if the fluid be then saturated with carbon dioxide, and the perfusion continued, part of the uric acid previously destroyed is re-formed.

Further, Ascoli and Izar,[3] in attempts to ascertain the nature of the chemical substances which were thus synthesised into uric acid, showed that by incubating liver extracts (ox) kept saturated with carbon dioxide with urea and dialuric acid a synthesis of uric acid could be brought about, but not when the added substances were allantoin or allantoin and urea. It seems likely, therefore, that the uric acid first destroyed in the perfusion experiments was not converted entirely into allantoin, or it would have been impossible to re-form any of the uric acid. This agrees with my own observations that the uric acid destroyed by the dog's liver is only partially converted into allantoin.

Similar observations to those already carried out on dogs[1] have now been made with rabbits. The excised livers of large rabbits have been perfused with saline solution containing sodium urate; the uric acid was estimated before and after the perfusion, and the amount of allantoin produced determined. The methods employed were identical with those used in the previous experiments.

As in the case of the dog, it was found that when a surviving liver is perfused with Ringer's solution alone a small quantity of allantoin is produced from the liver. In rabbits, owing to the much smaller size of the liver, this is very small, not exceeding 2 mgm. per cent. of the fluid, even in a five hours' perfusion.

Experiment I. The liver of a rabbit weighing 3,650 g. was perfused for two hours with Ringer's solution. At the end of the experiment the perfusion fluid contained 1·2 mg. of allantoin per cent. This melted at 231° C.; mixed with an equal quantity of Merck's allantoin it melted at 231·5° C. It had the crystalline form of allantoin, was readily soluble in hot water, with difficulty in cold, and was almost insoluble in alcohol. Boiled with sodium hydrate it gave the glyoxylic reaction, its solution gave no precipitate with silver nitrate alone but a flocculent one on the addition of a trace of ammonium hydrate which redissolved with excess.

Experiment II. The liver of a rabbit weighing 4,300 g. was perfused with Ringer's solution for five hours. At the end of the experiment the perfusion fluid contained 2 mg. of allantoin per cent. This melted at 226° C.; mixed with an equal part of Merck's allantoin and using the same thermometer the melting point was unchanged. It had the crystalline form and physical and chemical properties of allantoin.

When sodium urate was added to the perfusion fluid a considerable amount of the uric acid disappeared, even in such a short time as two hours, while in five hours only traces of uric acid may be left. The uricolytic power of rabbit's liver seems to be much greater, weight for weight, than that of a dog. The allantoin isolated from the fluid after perfusion corresponded to 85 per cent. to 100 per cent. of the uric acid which had disappeared. Under the conditions of these experiments, therefore, uric acid is converted quantitatively into allantoin by rabbit's liver, and it is most unlikely that any other substance is produced or that any allantoin is further destroyed.

Experiment III. The liver of a rabbit weighing 3,150 g. was perfused for two hours with Ringer's solution containing 48·8 mg. per cent. of uric acid as sodium urate. At the end of the experiment the fluid contained 30·6 mg. per cent. of uric acid and 19 mg. per cent. of allantoin. This melted at 231° C., and had the crystalline form and chemical and physical properties of allantoin. The percentage of nitrogen determined by Kjeldahl's method was 35·6 (theoretical 35·44).

In this experiment, allowing for 1·2 mgm. of allantoin produced from the liver itself (Exp. I), the whole of the uric acid destroyed was recovered as allantoin.

Experiment IV. The liver of a rabbit weighing 4,300 g. was perfused for two hours with Ringer's solution containing 33 mg. per cent. of uric acid as sodium urate. At the end of the experiment the fluid contained 6·9 mg. per cent. of uric acid and 22 mg. per cent. of allantoin. This had a melting point of 231° C. which remained unchanged when mixed with an equal part of Merck's allantoin. It had the crystalline form and physical and chemical properties of allantoin.

In this experiment, allowing a correction of 1·2 mgm. per cent., 85 per cent. of the uric acid destroyed was recovered as allantoin.

Experiment V. The liver of a rabbit weighing 3,220 g. was perfused for five hours with Ringer's solution containing 31·9 mg. per cent. of uric acid as sodium urate. At the end of the experiment the fluid contained 4·2 mg. per cent. of uric acid and 26·3 mg. per cent. of allantoin. This melted at 231° C. and the melting point remained unchanged when mixed with Merck's allantoin. It had the crystalline form and physical and chemical properties of allantoin.

In this experiment, allowing for a correction of 2 mgm. per cent. (Exp. II) of allantoin produced in five hours from the liver itself, 93·2 per cent. of the uric acid destroyed was recovered as allantoin.

Experiment VI. The liver of a rabbit weighing 2,800 g. was perfused for five hours with Ringer's solution containing 29 mg. per cent. of uric acid as sodium urate. At the end of the experiment the fluid contained 11·5 mg. per cent. of uric acid and 17 mg. per cent of allantoin. This melted at 231°C. when mixed with an equal part of Merck's allantoin it melted at 230°C. It had the crystalline form and solubilities and gave the chemical reactions of allantoin.

In this experiment, allowing a correction of 2 mgm. per cent., 91·4 per cent. of the uric acid destroyed was recovered as allantoin.

Conclusions

The excised liver of a rabbit, when perfused with Ringer's solution, produces a small quantity of allantoin. Sodium urate, added to the perfusion fluid, is converted quantitatively into allantoin. None of the allantoin so produced is further destroyed.

REFERENCES.

1. Ackroyd, *Bio-Chem. Journ.*, Vol. V, p. 217, 1910.

2. Bezzola, Izar and Preti, *Zeitsch. phsiol. Chem.*, Vol. LXII, p. 229, 1909.

3. Ascoli and Izar, *Zeitsch. physiol. Chem.*, Vol. LXII, p. 347, 1909.

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THE CHEMICAL DIFFERENTIATION OF SPECIES

By MURIEL WHELDALE, *Fellow of Newnham College, Cambridge.*

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The differentiation of species by the biologist is based upon structural characters. If resolution be carried sufficiently far, the units of such a system constitute the elementary species of de Vries.[1] Each unit differs from another in several or in a number of morphological characters and it is part of the qualification of a systematic unit that its characters, within the limits of individual fluctuation, should be fixed, and should be transmitted unchanged from parent to offspring.

From biological investigation, we also learn that the structure of a species is of necessity adapted to its environment. Though there is no satisfactory evidence in support of the view that environment takes active part in the modelling of the plant form, the latter, nevertheless, can be modified to some extent during the life-time of the individual by changes in the environment. Such modifications, however, are regarded as somatic, i.e., modifications in degree only of existing morphological characters, and are not transmitted through the germ cells in heredity. Hence, in this sense, environmental changes have no permanent effect upon a species.

On the other hand, new varieties, and we must suppose, new species too, which are in themselves true entities, may at any time arise from an existing species through the formation of a new kind of germ-cell. These new forms differ from the type in certain morphological characters and also transmit these characters to their offspring. Among causes instigating this kind of variation, change in environment may be included, though there appears to be no direct connection between the nature of the change and the kind of variation produced. The new variety or species may or may not be able to adapt itself to the environment into which it is cast.

In all probability, classification in the vegetable kingdom will always be based on structure only. At the same time we cannot fail to realise that the various structural characters of species are in reality the expression

1. de Vries, *Species and Varieties: Their origin by Mutation*, 1905.

of certain phenomena of which the interpretation lies in another sphere of scientific research. Hence increased knowledge in the field of morphology, however exact and comprehensive it may become, must, from the nature of its methods, fail to give an insight into the underlying causes of specific form. To gain this insight the plant should be viewed from the bio-chemical standpoint.

Advance in bio-chemistry brings increasingly to the fore the conception of a chemical basis for vegetable structure. Many, for instance, of the so-called 'vital processes' which were at one time considered to be special functions of the living protoplasm, are now known to be controlled by complex chemical substances termed enzymes; at the same time it cannot be denied that there still remains a residuum of activities produced by living cells which no enzymes, so far known, are able to effect. A considerable number of these enzymes have been isolated from the plant and their activities have been demonstrated in vitro. Although much remains to be learnt of the actual nature and structure of enzymes, yet such knowledge as we possess with regard to these substances, tends to bring the reactions they control into line with other chemical reactions with which we have long been familiar.

Not only has our knowledge of the mechanism of metabolism increased, but progress has also been made along the lines of artificial synthesis of 'vital' products and likewise in the isolation and exact analysis of hitherto unknown compounds present in plants.

All bio-chemical results of the above kind afford evidence in favour of the supposition that the plant form is an expression of its chemical constitution.

From a scientific standpoint it is easy to realise that the plant body may have various appearances according to the conditions under which it is viewed. Magnification, for instance, considerably alters our idea of its construction and leads the biologist to look upon the plant as a complex of tissues built up of morphological units or cells, some living and of a highly organised structure, others dead.

To the bio-chemist, the underlying reality of the plant is a complex of chemical substances, some in solution, others in a solid or crystalline state. In addition, one must picture many of these substances taking part continually in a number of very complex chemical reactions which are highly susceptible to environment, i.e., presence of moisture, light, degree of temperature, etc.

We know that one organic compound differs from another in the number, kind and configuration of its component atoms. On similar lines we may suppose the number, kind and configuration of the chemical compounds which go to build up a plant to determine the plant form, and differences in the chemical components to be the underlying cause of difference in plant forms. Such a conception helps us to realise how each plant maintains its specific entity. Each plant may be supposed to inherit from its parent a certain definite chemical constitution and to be able to build up, by virtue of this constitution, compounds of a certain chemical type only and these products in their entirety give the outward morphological manifestation peculiar to the species.

The modifications brought about in any species during its life-time by change in environment must be regarded as the expression of change, in degree only, in the chemical reactions possible to the metabolism of that species. The morphological character, pigmentation, affords examples of varying degrees of reaction due to change in environment. The leaves of a species, for instance, growing in a warm temperate climate, may normally show only a trace of red colour, because at that temperature the concentration of the products of metabolism does not permit the chemical reaction leading to pigment formation to take place to any extent. When transferred to a cold temperate climate, the leaves may become completely red, since the concentration of the metabolic products is now different and pigment formation is accelerated.

The origin of a variety must, on the other hand, be regarded as the expression of a fundamental change in the chemical constitution of the plant, whereby certain chemical reactions cease to take place and their resultant products to be formed, or new reactions are instigated and new products are produced. Thus a permanent change may be supposed to have taken place in the chemical constitution of a green-leaved type when it gives rise to a red-leaved variety, for example, Copper Beech. In the metabolism of the variety it is no longer possible for the chemical reaction inhibiting the formation of pigment to take place. Similarly in certain glabrous white-flowered varieties of *Matthiola*,[1] some portion of the chemical structure of the hairy purple-flowered type is lost and this loss is accompanied not only by total inability to form pigment but also by complete absence of a structural character, i.e., the outgrowth of hairs from the epidermal cells.

1. Bateson, Saunders, and Punnett, *Reports to Evolution Committee of the Royal Society*, I, II, and III.

In view of the fact that the structure of all the higher plants has, on the whole, a common basis, we should expect and do as a matter of fact find, that metabolism is conducted on the same general plan, and hence the main classes of plant products, i.e., proteins, carbohydrates, fats, aromatic compounds, etc., are represented in the majority of species.

It is also a well-recognised phenomenon that the same special morphological tendencies appear in different genera by no means closely allied. The herbaceous, woody, annual and perennial habits are characteristic of various members of a number of families among the higher plants. So also xerophytic, aquatic, saprophytic and parasitic forms appear throughout the vegetable kingdom. On analogy we may expect very similar or identical chemical compounds to appear in plants which are by no means closely related. Finally, if the differentiation of species is to be ultimately a matter of the configuration of the complex molecules which bring about what have been termed 'vital processes,' we should expect not only a different outward configuration but also the presence in different species, and groups of species, of characteristic types of chemical compounds.

That many chemical compounds are peculiar to certain species becomes obvious when an examination is made of any bio-chemical literature in connection with a group of organic compounds. There exists, in fact, a vast amount of knowledge of the chemical components of many species. The object of this kind of investigation has certainly not been the establishment of chemical differentiation, but in the majority of cases research has been stimulated owing to the fact that the substances investigated were used for medicinal or various commercial purposes, as, for example, the alkaloids, tannins, resins, oils, various vegetable dyes, etc. There has been very little application of these results in the direction of ascertaining whether any isolated plant product is peculiar to certain species only, to all species of a genus or to allied genera of a natural order. The differentiation of species upon a purely chemical basis would require a store of information which we are at present very far from possessing. I should like to take advantage of this opportunity to advance a plea for the application of such knowledge as we already have, or are likely to obtain in the future, to the furtherance of this scheme.

The investigations might be carried out along certain lines such as the following:—

(1) In case of isolation of a new substance, that its distribution among closely allied species and genera should be ascertained.

(2) That the distribution of known compounds which can be readily detected should be further investigated with reference to allied species and genera.

(3) That experiments should be made to detect as far as possible the various steps in the syntheses of plant products taking place in metabolism.

(4) That the knowledge we already possess of organic compounds occurring in plants should be co-ordinated with a view to the chemical differentiation of species.

As an example of systematic work of this kind, I may quote the case of the glucoside, *aucubine*. This substance was first isolated by Bourquelot and Hérissey[1] from the vegetative organs of *Aucuba japonica* (Cornaceae). Further researches by Hérissey and Lebas[2] demonstrated the presence of aucubine in several species of *garrya*, another genus of the Cornaceae. Aucubine is also said by Bourdier[3] to be present in various species of *Plantago* (Plantaginaceae). It would be interesting to discover whether further investigation will provide any connecting links between the two, so far isolated, appearances of this compound.

In the latter half of this paper I have attempted to give an account of the distribution of a few of the various classes of organic compounds found in plants and to show as far as possible (1) that many compounds are peculiar to certain species, genera or natural orders; (2) that allied compounds of restricted distribution are often found in the same species or genus.

I have dealt at greater length with the aromatic series because this group is universally present in plants and yet shows a greater specificity in the distribution of many of its members than is shown in the case of other widely distributed classes, such as the sugars and acids of the aliphatic series.

1. Bourquelot et Hérissey, 'Sur l'aucubine, glucoside de *l'Aucuba japonica*,' *Annales de chimie et de physique*, 1905.

2. Hérissey et Lebas, 'Présence de l'aucubine dans plusieurs espèces du genre Garrya,' *Jour. Pharm. Chim.*, 1910.

3. Bourdier, 'Recherche biochimique des glucosides dans le plantain (aucubine) et dans la verveine (vérbenaline),' *Thèse doct. Univ.* (*Pharm.*), Paris, 1908.

Phenols

The *pyrocatechin* $C_6H_4(OH)_2(1:2)$ complex exists in many substances widely distributed in plants, and it is highly probable that the free product occurs more frequently than is generally supposed. Both Grafe[1] and Weevers[2] are of the opinion that the oxidation of pyrocatechin is the cause of the darkening of the organs of many plants on injury or after death.

Hydroquinone, $C_6H_4(OH)_2$ (1:4), is more limited in its distribution than pyrocatechin. As a glucoside—*arbutin*—it is characteristic of many genera in the Ericaceae and Pyrolaceae. Its presence also in *Pyrus communis* and *Protea mellifera* shows that it is not strictly limited to the above orders. In some recent researches, Bourquelot and Fichtenholz[3] have obtained most interesting results in connection with hydroquinone. These authors find 'that *Pyrus communis* alone contains arbutin, while none could be detected in *Cydonia vulgaris*, *Malus communis*, *Sorbus auenparia* or *S. torminalis*, all of which were formerly classified as *Pyrus*, but are now regarded by most botanists as distinct. There are therefore recognisable chemical characters correlated with the botanical characters, and the modern classification is justified on bio-chemical, as well as on morphological, grounds.'

Phloroglucin, $C_6H_3(OH)_3$ (1:3:5), is the most widely distributed of all the phenols. The phloroglucin complex is present in a large number of aromatic compounds, and in addition, phloroglucin forms esters with acids of the aromatic series.

Aromatic Alcohols, Aldehydes and Acids

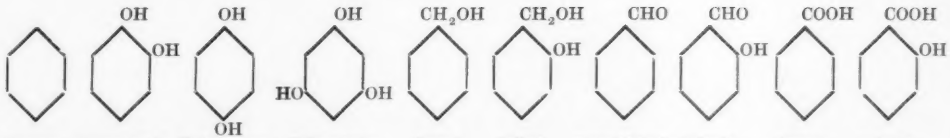
The relationships between the chief members of these derivatives, both in the benzene and the phenylolefine groups which are found in plants, are shown in the accompanying table.

Salicyclic alcohol in the form of a glucoside—*salicin*—is a characteristic product of the Salicaceae (*Salix*, *Populus*), but is not

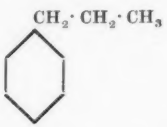
1. Grafe, 'Über die Dunkelfarbung von Rübensäften.' *Öst. Zeitsch. f. Zuckerindus. u. Landwirtschaft*, 1908.

2. Weevers, 'Die physiologische Bedeutung einiger Glykoside.' *Extrait du Recueil des Travaux botaniques Néerlandais*, Vol. VII, 1910.

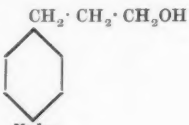
3. Bourquelot et Fichtenholz, 'Nouvelles recherches sur le glucoside des feuilles de poirier; son rôle dans la production des teintes automnales de ces organes,' *Jour. de Pharmacie et de Chimie*, 1911. See also *J.C.S. Abstracts*, February, 1911.



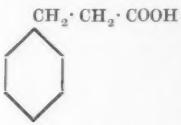
Benzene. Pyrocatechin. Hydroquinone. Phloroglucin. Benzyl alcohol. Salicyl alcohol. Benzaldehyde Salicylic aldehyde. Benzoic acid. Salicylic acid.



Propyl benzene.

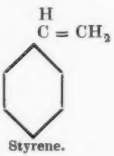


Hydro-cinnamyl alcohol.

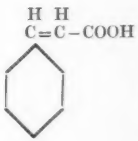


Hydro-cinnamic acid.

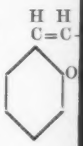
o. Hy coumar



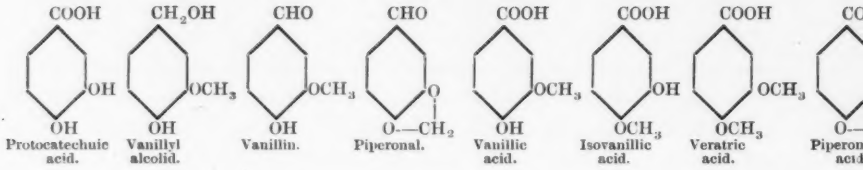
Styrene.

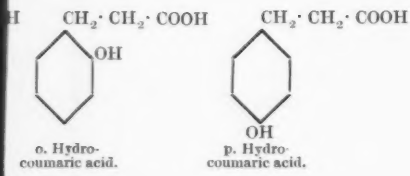


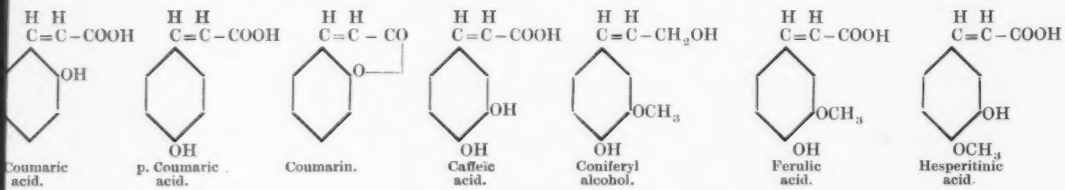
Cinnamic acid.



o. Coumaric acid.


COOH
OH
OH
Protocatechuic acid.
CH2OH
OCH3
OH
Vanillyl alcolid.
CHO
OCH3
OH
Vanillin.
CHO
O
O—CH2
Piperonal.
COOH
OCH3
OH
Vanillic acid.
COOH
OH
OCH3
Isovanillic acid.
COOH
OCH3
OCH3
Veratric acid.
CO
O—
Piperon
acid

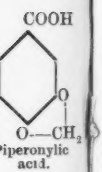

CH2· CH2· COOH
OH
o. Hydro-coumaric acid.
CH2· CH2· COOH
OH
p. Hydro-coumaric acid.


H H
C=C–COOH
OH
Coumaric acid.
H H
C=C–COOH
OH
p. Coumaric acid.
H H
C=C – CO
O
Coumarin.
H H
C=C–COOH
OH
OH
Caffeic acid.
H H
C=C–CH2OH
OCH3
OH
Coniferyl alcohol.
H H
C=C–COOH
OCH3
OH
Ferulic acid.
H H
C=C–COOH
OH
OCH3
Hesperitinic acid.

limited to this group, since it is said to occur also in Rosaceae (*Spiraea*), and in Compositae (*Crepis*).

Benzoic acid in the form of benzoyl salicin is found as a glucoside—*populin*—which is apparently limited to the Salicaceae.

Salicylic acid, either free, or in the form of a glucoside of the methyl ester, is very widely distributed:—Liliaceae (*Yucca*), Rutaceae (*Barosma*), Rosaceae (*Spiraea*, *Fragaria*, *Rubus*), Rubiceae (*Ipecacuanha*), Violaceae (*Viola*), Ericaceae (*Gaultheria*), Betulaceae (*Betula*), Pyrolaceae (*Monotropa*), Fagaceae (*Fagus*), Polygalaceae (*Polygala*), Lauraceae (*Lindera*) and Erythroxylaceae (*Erythroxylon*). *p. Oxy-benzoic acid* is found in Bignoniaceae (*Catalpa*).



Piperonylic acid.

That a metabolic relationship exists in the Salicaceae between salicylic alcohol and pyrocatechin has been shown by Weevers[1] in an interesting paper on glucosides. The author finds that a decrease of saligenin is accompanied by an increase of pyrocatechin, and vice versa. Weevers was also able to obtain three enzyme actions from 'Rohenzyme' extracted from *Salix*, though the enzymes were not isolated. One enzyme—salicase—brings about the reversible action: salicin + water = saligenin + glucose; the second—saligeninase—oxidises saligenin to pyrocatechin; and the third—catecholase—oxidises pyrocatechin to a black amorphous substance.

There is also obviously a relationship between the salicylic alcohol and salicylic acid in *Spiraea*.

Protocatechuic acid. The protocatechuic acid nucleus forms a constituent of many aromatic plant compounds, and the acid itself is formed as a product in the potash fusion of these substances. Native, the acid, occurs in Magnoliaceae (*Illicium*) and in Vitaceae (*Vitis*).

Vanillin occurs as a glucoside in Orchidaceae (*Vanilla*), and vanillin-producing substances are probably fairly widely distributed:—Orchidaceae (*Vanilla*, *Nigritella*), Graminaceae (*Avena*), Leguminoseae (*Lupinus*), Liliaceae (*Asparagus*), Chenopodiaceae (*Beta*), Aquifoliaceae (*Ilex*), and Coniferae (*Pinus*).

Piperonal accompanies vanillin in *Vanilla* and *Nigritella*, and it is also found in *Heliotropium* (Boraginaceae).

Veratric acid is only known to occur in *Veratrum* (Liliaceae).

The phenyl-olefine series may next be considered.

Cinnamic acid is widely distributed, though not very frequent:—

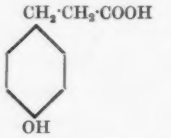
1. Weevers, *loc. cit.*

Erythroxylon (Erythroxylaceae), *Thea* (Theaceae), *Scrophularia* (Scrophulariaceae), *Globularia* (Globulariaceae), and *Alpinia* (Zingiberaceae).

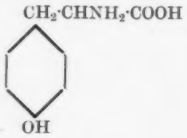
The phloroglucin ester of p-coumaric acid—*naringenin*—is present as a glucoside—*naringin*—in *Citrus decumana* (Rutaceae), and appears to be confined to that genus.

From the coumaric acids it is possible to pass to the phenyl fatty acids.

o-Hydrocoumaric acid has been found only in *Melilotus* (Leguminoseae). *p-Hydrocoumaric acid*, as pointed out by Czapek, is of interest because of its connection with tyrosine.



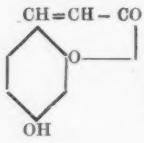
p. hydrocoumaric acid.



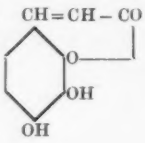
tyrosine.

Coumarin is a very widely distributed substance:—*Phoenix* (Palmaceae), *Anthoxanthum, Hierochloë, Milium, Cinna* (Graminaceae), *Angraecum, Nigritella, Orchis, Aceras* (Orchidaceae), *Herniaria* (Caryophyllaceae), *Ruta* (Rutaceae), *Prunus* (Rosaceae), *Dipteryx, Melilotus, Toluifera* (Leguminoseae), *Myroxylon* (Flacourtiaceae), *Vitis* (Vitaceae), *Peristrophe* (Acanthaceae), *Galium, Asperula* (Rubiaceae), *Ageratum, Liatris* (Compositae).

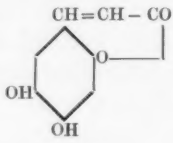
Several oxy-coumarins have been found in various plants:—



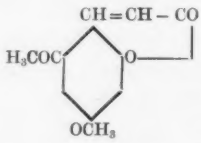
Umbelliferone



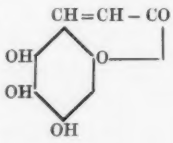
Daphnetin



Æsculetin



Limettin



Fraxetin

Umbelliferone is found in *Daphne* (Thymeleaceae), and probably also in *Skimmia* (Rutaceae). Its methyl ester is said to be present in *Herniaria* (Caryophyllaceae).

Daphnetin as a glucoside—*daphnin*—is confined to *Daphne* (Thymelaeaceae).

Æsculetin occurs as a glucoside—*aesculin*—in *Æsculus* (Hippocastanaceae), *Gelsemium* (Loganiaceae), and *Euphorbia* (Euphorbiaceae).

Scopolin, a methyl aesculetin glucoside, occurs in *Scopolia* and Atropa (Solanaceae).

Limettin is found in *Citrus* (Rutaceae).

Fraxetin accompanies aesculin in *Æsculus* (Hippocastanaceae), and is also found in *Fraxinus* (Oleaceae).

Caffeïc acid is probably widely distributed:—*Conium* (Umbelliferae), *Coffea*, *Chiococca* (Rubiaceae), *Ilex* (Aquifoliaceae), *Scrophularia* (Scrophulariaceae), *Strychnos* (Loganiaceae).

Coniferyl alcohol as a glucoside—*coniferin*—is a characteristic product of the Coniferae. It is found also in *Beta* (Chenopodiaceae), *Asparagus* (Liliaceae), *Scorzonera* (Compositae).

Syringenin, methoxy-coniferyl alcohol, occurs in *Ligustrum* and *Syringa* (Oleaceae), and also in *Robinia* (Leguminoseae).

Ferulic acid has been found in the resin of Asafœtida.

Hesperitinic acid as a phloroglucin ester—*hesperidin*—is a product of many Rutaceae, i.e., *Citrus*, *Barosma*, *Toddalia* and *Skimmia*, and is also found in *Scrophularia* (Scrophulariaceae).

When we consider the question of allied products, the following facts are of interest.

Coniferyl alcohol is readily oxidised to vanillic alcohol, though it appears probable that hadromal is the mother substance of vanillin. Nevertheless, both nearly related products do appear in the same genera.

Vanilla and *Nigritella* (Orchidaceae) contain vanillin, piperonal and coumarin.

Pinus (Coniferae), *Asparagus* (Liliaceae) and *Beta* (Chenopodiaceae) contain both vanillin and coniferyl alcohol.

Scrophularia nodosa (Scrophulariaceae) contains cinnamic, caffeïc and hespertinic acids.

Daphne, umbelliferone and daphnetin.

Ilex, vanillin and caffeïc acid.

Æsculus, aesculin and fraxetin.

Herniaria, umbelliferone and coumarin.

Melilotus, o-hydrocoumaric acid and coumarin.

In the Rutaceae, many substances allied to cinnamic acid are found, i.e., naringin, umbelliferone, coumarin, limettin and hesperitin.

Proteins

These substances form an essential constituent of every plant, yet of all classes of compounds they are probably the least adapted to purposes of chemical differentiation. To quote Osborne on 'The Vegetable Proteins':—'The possibility of making preparations of proteins which may fairly be considered as chemical individuals deserves special attention. These substances present unusual difficulties in this respect, for they have none of those physical and chemical characters which the chemist usually depends upon to show the individuality of an organic chemical compound; in other words, the proteins have no single property by means of which any judgment can be formed in regard to the strict chemical individuality of any one of them. The best that can be done at present is to establish a constancy of the ultimate composition of successive fractional precipitations of the protein under consideration, and to show the constancy of the physical properties and products of hydrolysis of these fractions as far as possible . . . . Many, however, of the proteins with which we are at present familiar have shown such constancy of composition and properties that we feel justified in now considering them as substances of reasonably definite character.'

There is, nevertheless, evidence for regarding many of the known proteins as distinctive of certain species or groups of species. Definite names, for instance, have been given to the following globulins:—

Leguminoseae: legumin in *Pisum sativum, Vicia faba, V. sativa, Ervum lens;* vignin in *Vigna sinensis;* glycinin in *Glycine hispida;* phaseolin, *Phaseolus vulgaris, P. radiatus, P. lunatus;* conglutin, *Lupinus* sp; vicilin, *Pisum sativum, Vicia faba,* and *Ervum lens.*

Corylaceae: corylin in *Corylus avellana.*

Rosaceae: amandin in *Prunus amygdalus, P. persica.*

Juglandaceae: juglansin in *Juglans regia, J. nigra, J. cinerea.*

Lecythidaceae: excelsin in *Bertholletia excelsa.*

Cannabinaceae: edestin in *Cannabis sativa.*

Graminaceae: avenalin in *Avena sativa;* maysin in *Zea mays.*

Fagaceae: castanin in *Castanea vulgaris.*

Solanaceae: tuberin in *Solanum tuberosum.*

The Mustard-Oils

The mustard-oils, in combination with sugar as various glucosides, form as one group—sinigrin, sinalbin, sinapin, etc.—characteristic components of the Cruciferae, and as a second group—glukotropaeolin—of the Tropaeolaceae.

Purin Derivatives

Caffeïne: *Coffea* (Rubiaceae).
Thea (Theaceae).
Paullinia (Sapindaceae).
Ilex (Aquifoliaceae).
Cola (Sterculiaceae).
Theobroma (Sterculiaceae).

Theobromine: *Theobroma* (Sterculiaceae).
Cola (Sterculiaceae).

Theophylline: *Thea*.

Xanthine: *Thea*.

Adenine: *Thea*.

As will be seen from the above list, several of these compounds (theophylline, xanthine and adenine) are known only in the genus *Thea* (Theaceae). Theobromine in *Theobroma* and *Cola* (Sterculiaceae). Caffeïne is more widely distributed.

Cyanogenetic Compounds

As glucosides, these are characteristic products of certain genera. *Amygdalin* and *laurocerasin* are found in genera of the Rosaceae, but there is evidence that amygdalin-like compounds are widely distributed among plants. Other cyanogenetic glucosides are *dhurrin*, peculiar to *Sorghum* (Graminaceae); *gynocardin*, to *Gynocardia; phaseolunatin*, to *Phaseolus lunatus* (Leguminoseae), and *Linum* (Linaceae); *lotusin*, to *Lotus arabicus* (Leguminoseae).

Alkaloids

The alkaloids form a group of organic substances specially characterised by the fact that every member of the group, with a few exceptions, is a product of metabolism in some one species only, in one genus only, or at most in several allied genera.

Considering the group as a whole, we find the alkaloids especially prominent in certain orders and cohorts, whereas they are entirely absent from others. The Monocotyledons are poor in alkaloids. Among the Dicotyledons, the chief alkaloid-containing groups are the Ranales (Ranunculaceae, Menispermaceae, Berberidaceae, and others); the Rhoeadales (Papaveraceae); the Contortae (Loganiaceae, Apocynaceae); the Solanaceae; the Rubiales (Rubiaceae).

From other large families of flowering plants, these substances are either entirely absent (Rosaceae, Labiateae), or are present in one or two genera only (Cruciferae, Umbelliferae, Compositae).

The alkaloids are generally classified into three groups, according to whether they are derivatives of pyridine, quinoline, or isoquinoline. Derivatives of each group are represented in various natural orders.

The pyridine group is mostly widely represented. The best known derivatives occur in the Solanaceae, Erythroxylaceae, and Leguminoseae, but other members are present in a large number of orders, including Palmaceae, Liliaceae, Amaryllidaceae, Piperaceae, Zygophyllaceae, Rutaceae, Euphorbiaceae, Buxaceae, Celastraceae, Caricaceae, Cactaceae, Punicaceae, Myrtaceae, Umbelliferae, Caprifoliaceae, Cornaceea, Plumbaginaceae, Sapotaceae, Symplocaceae, Apocynaceae, Boraginaceae, Verbenaceae, Campanulaceae, Cucurbitaceae, and Compositae.

Members of the quinoline group are chiefly characteristic of the Loganiaceae (*strychnine* and *brucine*, and other derivatives in *Strychnos* spp.), and of the Rubiaceae (*quinine* and *cinchonine*, and other derivatives in *Cinchona* spp.).

Members of the isoquinoline group are found in Nymphaeaceae, Ranunculaceae, Berberidaceae, Anonaceae, Menispermaceae, Calycanthaceae, Lauraceae, and Papaveraceae.

It is rare to find an alkaloid distributed among the genera of more than one natural order. *Trigonellin*, which is present in the Leguminoseae, Moraceae, Graminaceae, Apocynaceae, and *berberin* in the Ranunculaceae, Berberidaceae, Anonaceae, Menispermaceae, Leguminoseae, and Rutaceae, both form examples of this kind of exception.

R. Faulkner & Co., Photographers, 21 Baker Street, London.

SIDNEY RINGER

Mau

IN MEMORY OF SIDNEY RINGER

[1835-1910]

SOME ACCOUNT OF THE FUNDAMENTAL DISCOVERIES OF THE GREAT PIONEER OF THE BIO-CHEMISTRY OF CRYSTALLO-COLLOIDS IN LIVING CELLS

It is often notable, on consulting the original writings of master-workers in any domain of knowledge, to what an extent these contain, crystallized out in single sentences, or short paragraphs, discoveries of great beauty or intense importance, which have afterwards been re-discovered and related at ream's length by subsequent writers.

This observation will strike anyone who cares to dive into the original papers of Thomas Graham on colloids, and it is no less striking in the writings of his colleague, Sidney Ringer, on the correlated subject of the crystallo-colloids and the balanced activities of the inorganic constituents in regulating and controlling the energy discharges of living cells.

Both physical chemists and bio-chemists have nowadays come to realise that the most fruitful ground of both chemistry and biology lies in the land of colloids, and of colloidal and crystalloidal relationships. It is a remarkable fact that the master-discoveries in these two closely related domains belong to two men identified with the same institution, who, keen though they were over their experiments, dimly, if at all, realised the vast extensions which lay before their discoveries. Graham does appear to have dimly seen that there was a relationship between the energetics of the colloidal state and the life processes of cells, and draws attention to the fact that colloids are unstable and all the time suffering slow energy changes, while crystalloids are inert and inactive in stable equilibrium.

Ringer equally appreciated the importance of the inorganic salts in living processes, in stimulating or stilling muscular activity, or in varying the velocity of growth and repair in living cells of all types.

But the state of advancement of knowledge at the time the discoveries were made precluded either observer from surveying the whole field and seeing how much all these researches were complemental to one another, and that the ultimate substrate for the energy display was the delicately balanced equilibrium between colloid and crystalloid.

Ringer's researches, which yield such a magnificent demonstration of the applicability of the fundamental principles of the ionic theory, were made independently of it, and before it had gained any general acceptance. Accordingly, one can only marvel now at the experimental inspiration and clear logical following out of sequences which enabled him to develop one fact after another, track out one possibility after another, and eliminate everything extraneous until he had found the true connection between the phenomena with which he was dealing.

Remarkable accuracy in observing detail, thoroughness in investigating every factor in the problem, however trivial at first sight that factor might seem, and untiring energy in repetition of experiment until he was quite certain he held the whole truth of the case without omission or exaggeration, were the traits which went to make up Ringer's character, both as a scientific observer in the laboratory and as a clinician in the hospital. At the active period of his scientific work he was a most prolific writer, yet all his work, so far as he was responsible, has stood the test of time and grown in beauty in men's eyes with the lapse of the years.

In one of his later conceptions only was Ringer misled by a circumstance over which he had no control. The laboratory supply of distilled water contained an unsuspected impurity, viz., a trace of copper, due to a defect in the still, and this misled Ringer as to the action of the supposed pure distilled water upon living organisms. The writer happened at the time to be working in collaboration with him at another piece of work, and, at his request, tested the laboratory water; he can still well remember Ringer's chagrin and disappointment when the presence of copper in the water was demonstrated to him. This incident, although he could not possibly have foreseen it, troubled him not a little; he temporarily ceased work upon his beloved subject, and other events which meanwhile occurred in private life conspired to prevent him ever afterwards taking up laboratory work with his wonted energy, so that he never contributed further to the subject, with the

exception of a short note to the Physiological Society, published about two years later.[1]

The first of the remarkable series of papers which record Ringer's discoveries of the actions of inorganic salts (or ions) upon the beat of the heart, is contained in the third volume of the *Journal of Physiology*, 1881-2, under the title 'Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle.' This first paper demonstrates clearly Ringer's scientific insight and doggedness of purpose. He shows that certain changes in the ventricular beat, which, at this stage, he thinks are due to normal saline (0·75 per cent. solution of sodium chloride), but which in a later paper he finds out are due to a trace of calcium salt in the supply of New River Water with which the saline has been made up—he shows that these changes can be obviated if a certain amount of dried blood is added to the saline. Proceeding further, he finds that diluted white-of-egg added to the saline produces a like result in maintaining the normal heart-beat. The ordinary investigator would probably have stopped here, thinking he had done well and probed matters to the limit. Not so Ringer, and this is precisely how he made the first step that counts so much towards his great discovery. Having discovered that both dried blood extract and diluted egg-white act upon the contracting ventricle, he does not jump to

1. This note takes up the matter of the supposed action of distilled water on Tubifex, and is characteristic of Ringer, both in its carefulness and exactitude and in the candour and honesty with which it admits the truth of Locke's position: that the supposed poisonous and disintegrating action of distilled water obtained by distillation from a metal still and condenser are due to *infinitesimal* traces of heavy metals in the water. He first of all re-distilled from glass thirty litres of the type of water used originally with poisonous effects, and now found the distillate harmless, and the residue to contain copper and be violently poisonous until after exposure to air for some days. Then he collected Nature's own distillate in the form of rainwater on his Yorkshire moorside in earthenware vessels, and demonstrated that this water was harmless to the organisms although it contained no trace of calcium salts. On placing a few small pieces of copper filings in this innocuous water it became poisonous and disintegrated Tubifex in a few hours. As a result of this careful enquiry he frankly admits that: 'These experiments appear to me to establish Locke's conclusion that copper in even infinitesimal quantities will disintegrate Tubifex, whilst water free from copper or other heavy metals, and without any salts such as calcium salts, can sustain the life of Tubifex.' In spite of this disappointment it is interesting to observe Ringer's mental activity in seizing the opportunity to demonstrate to the hilt the most important fact which he had earlier observed as to the antagonism of the potassium and calcium effects. He says, 'I next tested with this rainwater from the moors the antagonism between potassium chloride and calcium chloride. I placed Tubifex in a 0·1 per cent. solution of potassium chloride, in about four hours they were almost motionless, and with the microscope I was unable to see the vessels contract; they appeared quite paralysed except just below the head of the animals. I then placed the Tubifex in a 0·1 per cent. KCl and 0·1 per cent. $CaCl_2$ and almost at once spontaneous movement increased, and in five minutes they became quite active. Seven hours later they were moving energetically; before movement was so much restored the microscope showed that the vessels, even to the tail were contracting vigorously.' (*Proc. Physiol. Soc.*, December, 1897, *Journ. of Physiol.*, Vol. XXI, pp. 14, 15.)

what, at that stage of our knowledge, would be the most obvious conclusion, viz., that this common good result arose from the nutrition of the contracting ventricle by the proteins common to both fluids. Here are the discoverer's own words on the subject:—'Blood, then, obviates the changes occurring in the contractions of a ventricle receiving only saline solution, and the question arises, which constituent of the blood has this property?'

'I find that a small quantity of white-of-egg completely obviates the changes occurring with saline solution. I first took tracings with blood mixture; I then replaced the blood with 100 c.c. of saline, and got the usual great prolongation of dilatation. I then added to the saline 25 c.c. of white-of-egg mixture, composed of one part white-of-egg in two parts of water. In two minutes the contractions became exactly as they were when the heart was supplied with blood mixture. White-of-egg consists of albumins and chloride of potassium and sodium, chiefly chloride of potassium. It is obvious in the preceding experiment that the effect of white-of-egg could not be due to the sodium chloride. The power to obviate the great prolongation of the dilatation occurring when the ventricle was supplied with saline solution must, then, be due to the albumins or the potassium chloride.'

'I find that potassium chloride in small quantities, much smaller than exists in serum, will completely and speedily obviate the character of the trace occurring with saline solution, and give a trace in all respects like that occurring when the ventricle is supplied with blood mixture.' [References to the tracings here.] 'I first took a tracing with blood mixture, and then replaced the blood with 100 c.c. of saline solution. After about 15 minutes I obtained the usual great prolongation of the ventricular dilatation. I then added to the 100 c.c. of circulating saline 0·7 c.c. of 1 per cent. solution of potassium chloride at the point indicated by the arrow; very speedily the contraction became modified, the dilatation becoming much shorter. Ten minutes after the addition of the potassium chloride the contraction became just like those at the beginning of the experiment when the ventricle was supplied with blood. From numerous experiments, I find that from 0·6 c.c. to 1 c.c. of 1 per cent. solution of potassium chloride to the 100 c.c. of saline solution is sufficient to remove the prolongation of dilatation occurring with saline solution.'

At the date of this paper the ionization theory of Arrhenius had not yet appeared on the scientific horizon, and hence it is most interesting to

observe Ringer, in complete ignorance of it of course, proceeding to accumulate evidence in strong support of it had it only been known later to the supporters of the theory in their days of battle.

In the same manner, as recounted in his own words above, Ringer proceeded to test the actions of phosphate of potash and phosphate of soda (as he calls them), and found that phosphate of potash had precisely the same action as potassium chloride, while phosphate of soda had no action. His conclusion is that, 'the effect, then, in potassium phosphate is due to the potassium and not to the phosphoric acid.'

'Probably any potassium salt is sufficient to obviate the prolonged dilatation occurring when the ventricle is fed with saline solution only, for I have produced normal contractions by adding to the saline solution potassium chloride, potassium phosphate, potassium sulphate, potassium citrate, potassium bi-carbonate, potassium chlorate, potassium nitrate, potassium acetate, and potassium carbonate.'

Throughout this paper Ringer is ignorant of the fact that the prolongation of ventricular relaxation antagonized by the potassium salts is due to the presence of an unbalanced trace of calcium salt in his saline solution. It is a remarkable piece of scientific good fortune that this accident had occurred, for although the diminution in force of beat and stoppage in pure distilled water *plus* sodium chloride is characteristic enough as compared with the full 'Ringer's effect' as now known to us, yet any such slowing down without the long tailing off produced by unbalanced calcium ion would probably not have attracted Ringer's attention in these earlier days, or called forth his energies to the same extent.

In his next paper, however, in the fourth volume of the *Journal of Physiology*,[1] he has discovered the great influence of minute traces of calcium upon the heart. Previous work of his own and of others upon the antagonistic action upon the heart of different alkaloids had prepared his mind, and the thought of an antagonism, as he calls it, between calcium and potassium fell upon prepared ground. In this second paper he clearly shows how different inorganic constituents can balance or antagonise one another, and this is reiterated in paper after paper of the succeeding members of the long series, and upon one type

1. The papers are not dated in these earlier volumes of the *Journal of Physiology*, and it is somewhat difficult to accurately assign dates; the paper immediately preceding Ringer's is dated November, 1882, so the date must be the end of 1882, or early in 1883. A later reference given by Ringer himself in Vol. XVI of the *Journal of Physiology* places these earlier papers in 1883 (See *Journ. Physiol.*, Vol. XVI, p. 1).

of material after another, such as ventricular muscle, skeletal muscle, growing frog's eggs, tadpoles, small fishes, tubifex worms, and so on. It is indeed remarkable that any man should have so reiterated and driven home a discovery of such first-rate importance, himself recognising its great value, although naturally he did not realise its more modern extensions, and that then for years he should be almost unknown in connection with it, save perhaps as the inventor of 'Ringer's solution.'

The description, in the discoverer's own words, of how he came upon the calcium effect is highly interesting. He says:—'After the publication of a paper in the *Journal of Physiology*, Vol. III, No. 5, entitled "Concerning the influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle," I discovered that the saline solution which I had used had not been prepared with distilled water, but with pipe water supplied by the New River Water Company. As this water contains minute traces of various inorganic substances, I at once tested the action of saline solution made with distilled water, and I found that I did not get the effects described in the paper referred to. It is obvious, therefore, that the effects I had obtained are due to some of the inorganic constituents of the pipe water.'

He then quotes a detailed analysis of New River Water, showing that amongst other things it contains 38·3 parts in a million of calcium, and proceeds experimentally to investigate calcium effects upon the ventricle. He shows that saline made from distilled water with pure sodium chloride does not give the rounded top and slow tailing off, but only shows a gradual cessation of activity altogether; then he adds a trace of calcium salt and obtains the now well-known result previously given by the New River Water saline. Further addition of the necessary small amount of a potassium salt takes away the slow relaxation and gives a normal beat which does not diminish and cease like that in presence of sodium chloride only, but remains strong and good for hours. After much experimentation and elimination or due appraising of actions of different constituents, Ringer describes the separate and combined effects as follows:—

'The heart's contractility cannot be sustained by saline solution, nor by saline containing potassium chloride, nor with saline solution containing bicarbonate of soda, nor by saline solution containing bicarbonate of soda and potassium chloride; but after contractility has ceased, the addition of a lime salt will restore good contractility. The addition, too, of a calcium salt to any of the above solutions will sustain

contractility. I conclude, therefore, that a lime salt is necessary for the maintenance of muscular contractility.' 'But while calcium salts are necessary for the proper contraction of the heart, yet if unantagonised by potassium salts the beats would become so broad and the diastolic dilatation so prolonged that much fusion of the beats would occur and the ventricle would be thrown into a state of tetanus.' 'As the ventricle will continue to beat perfectly for hours without any sodium bicarbonate, it is evident that the normal trace is the result of the antagonising action of calcium and potassium salts.' 'If these two salts [we should say ions at the present day] are not present in the correct proportions, then the trace becomes abnormal. If too little potassium is present, the contractions become broader, etc., and there results fusion of the beats. If too much potassium is present, or too little lime salts, then the contraction of the ventricle is imperfect, and by increasing the quantity of potassium salt the beat becomes weaker and weaker till it stops.' 'As the heart will continue to beat quite normally for hours without albumen or haemoglobin, it is obvious that these substances are not immediately necessary for contraction, but of course they are necessary to reconstruct the tissues from the loss due to contractions.'

In these few sentences we have the whole law and gospel of the action of inorganic constituents on contractile tissue, and since then there has been chiefly adding of detail. A great deal of detail was added in this and later papers by Ringer himself, and he demonstrated the same relationship in the excitation of rhythmic contractions in skeletal muscle and their inhibition by the antagonistic salts. In the next paper Ringer tests the effects of an excess of potassium salts, and finds that the latent period is greatly prolonged, and also the period of diminished excitability, so that the frequency of beat becomes diminished. In this paper also he points out a remarkable change to faradic stimulation of the ventricle induced by potassium. In absence of any excess of potassium, faradization causes a fusion of beats in a contracted or systolic condition which Ringer, following Marey, speaks of as tetanus; in presence of an excess of potassium there is a reverse action, for now faradization completely arrests the contractions which recommence on discontinuing the faradization,

Rubidium in all respects acts similarly to potassium, but the higher member of the alkali group, viz., caesium, possesses a quite different action.

'Caesium, indeed, produces for the most part the opposite effects to

potassium, broadening the trace, rounding its top, inducing fusion; yet in spite of this great difference in its action, still caesium, like potassium, possesses the property of obviating the effect of calcium on the dilatation.' He finds that calcium and caesium make a fairly good balance; but strontium and caesium will not make a good working pair. Strontium and potassium work; but not barium and potassium. The separate action of caesium resembles that of barium, but barium and calcium do not balance as do caesium and calcium.

'It is interesting to compare the effect of chemical similarity on physiological similarity in the group calcium, strontium, and barium, and in the group including potassium, rubidium, and caesium.'

'Strontium is chemically more allied to calcium than barium is to calcium, and whilst strontium is in its physiological action very similar to calcium, barium is far less similar to calcium. So it is with the group potassium, rubidium, and caesium. Rubidium is chemically more allied than caesium to potassium, and rubidium is almost identical in its physiological action with potassium, whilst caesium differs in most of its physiological effects from potassium.'

The effects of saline media on fishes are discussed in a communication to the Physiological Society in December, 1883, and in a most interesting paper appearing a few months later in the *Journal of Physiology* (Vol. V, p. 98). In these researches much interesting matter is brought together as to the behaviour of both fresh-water and salt-water fish when subjected to variations in the salinity of the medium in which they are living. It is demonstrated by Ringer that even fresh-water fish, such as minnows and sticklebacks rapidly perish when kept in distilled water,[1] and the effects of variations in oxygen supply being ruled out by experiment, it is shown that sodium, potassium and calcium salts are necessary to life in water. He shows that calcium in small amounts greatly prolongs life in distilled water, and that additional increase of longevity is obtained when sodium carbonate and potassium chloride are also added.

The concentration of calcium necessary for the sustenance of life is very low, but this can be exceeded manifold without causing the death of the fish; thus, as much as 0·5 per cent. of calcium chloride in the water could be borne by minnows.

In the next paper Ringer proceeds to examine the effects upon heart

1. In view of later discoveries it would now be of interest to repeat these earlier experiments of Ringer on the action of distilled water on organisms.

muscle of large toxic doses of calcium and potassium in the unbalanced and balanced conditions, and shows that a dose of either salt (or ion) which would be many times sufficient to poison the ventricle can be borne without ill effects when balanced by its antagonist. The paper might rank as a study of a balanced reaction at the present day, now about twenty-six years later. The whole subject illustrates the balancing of toxin and anti-toxin, as shown with inorganic ions. The calcium and potassium ions do not unite in stochiometric relationships with one another; in fact they have no appreciable chemical affinity for each other; but both are claimants for the bio-plasm of the ventricular muscle-cells, and in proper proportions determined by their chemical coefficients for the bio-plasm, we get balancing and the to-and-fro beat of the heart. This ratio can evidently exist at many corresponding concentrations of potassium and calcium, but a balance must be maintained. To each concentration of either of the two, there is a short range within which the other ion must lie.

The next paper, also in the fifth volume of the *Journal of Physiology*, demonstrates that veratria behaves similarly to calcium upon the heart ventricle, and can even replace lime salts, though somewhat imperfectly. The interesting fact is also shown that veratria and potassium, just like calcium and potassium, are mutually antagonistic.

In a second paper, dealing also with veratria and potassium, Ringer attempts some analysis of the different molecular reactions, as he terms them, occurring during contraction, relaxation, and reparation in the ventricular tissue. His words show a remarkable insight for the period at which they were written, and historically are worthy of quotation.

'In a muscular contraction three distinct forms of molecular change are obvious; namely, the changes occurring during the latent period, the changes which occur during contraction, and the changes causing the dilatation.' 'Those molecular changes which lead to dilatation are generally considered restorative or reparative; they prepare new material for the ensuing contraction, or remove waste products which would retard or prevent contraction; or they consist of both of these processes combined.' 'The independence of those molecular changes which occur at the termination of a contraction (and which cause dilatation) from the reparative changes is well shown by the action of lime on the ventricle. Calcium chloride, added to saline solution, greatly delays dilatation, but does not in any degree delay the reparative changes, so that no matter at

what period the excitation is made, the succeeding contraction is complete, even though the excitation is applied before dilatation is finished. Here, then, we have a drug which, like veratria, retards those molecular changes that lead to dilatation, but, unlike veratria, in no degree lessens the rapidity of the reparative changes.'

'We have seen that both veratria and potash salts retard the reparative changes, veratria more so than potash, and yet when these substances are both added to the circulating fluid they do not in this respect intensify each other's action, but distinctly antagonise each other, the reparative changes occurring much more rapidly after the addition of potash to the veratrized circulating fluid. We have, then, another instance of drugs having a common action, and yet when both are conjointly added to the circulating fluid, one antagonises the other in respect of this common effect.'

In a somewhat lengthy paper entering into a discussion of contemporary continental opinion as to whether organic nutrition is directly necessary to the heart-beat, Ringer attempts to separate all salts by dialysing albumin solutions, but finds it impossible to get rid of the calcium which adheres after prolonged dialysis. He then shrewdly suspects what we now know to be the correct view, that it is this strongly adherent calcium which enables albumin solutions to keep up contractions without the addition of inorganic calcium salt.

The next contribution is a most interesting one on the effect of lime salts upon the absorption of water by laminaria and other organic structures. It is full of interesting observations, the details of which cannot be entered upon here; but it is well worthy of perusal to-day by anyone interested in the modern problems of absorption of salts by colloids.

In a set of three papers following rapidly on this, Ringer demonstrates most clearly the action of calcium and other ions upon *skeletal* muscle. The first of the series is by Ringer alone, and in it the muscle is simply immersed in the various saline solutions. In a later paper, in collaboration with Dudley Buxton, the similar effects are described which are obtained when the various solutions are perfused from the aorta.

These papers are of great historic interest to all students of the action of inorganic ions on living cells, and since Ringer's connection with this work appears to have been well nigh forgotten, some recognition may here be made by quoting his main findings from the

earlier paper, and reproducing certain of his tracings.[1] The paper is contained in the *Journal of Physiology*, Vol. VII, pp. 291-308, 1886. It is noteworthy that it was here that Ringer first commenced to use calcium phosphate in his circulating fluid, having previously used chlorides only of sodium, potassium and calcium, that is up till this stage, he had himself always used what is now a modern modification of Ringer's solution. It is also of interest that his calcium phosphate, as he tells us, was analysed for him by his colleague Dr. Graham, Professor of Chemistry at University College. After exhaustive experiments on the periods during which skeletal muscles kept in various saline combinations retained their direct faradic excitability, he classifies them in the following order and then proceeds to describe his experiments on the contractions of skeletal muscle in saline solutions:—

'We see then that these solutions in respect of their efficiency to sustain contractility stand in the following order. Saline solution is the least effective, next follows saline containing sodium bicarbonate, then phosphate of lime saline, next phosphate of lime saline containing potassium chloride. Phosphate of lime saline containing potassium chloride and phosphate of lime saline containing potassium chloride and sodium bicarbonate sustain contractility about an equal time. Phosphate of lime saline sustains contractility longer than saline solution containing sodium bicarbonate.'

'Now this being the order of their efficiency as circulating fluids for the heart, it would appear that lime and potash salts are as necessary to the metabolism of muscle at rest as to the metabolism occurring during a muscular contraction.

Another circumstance however explains in part the efficacy of lime and potassium salts in sustaining contractility in quiescent muscle.

I noticed in the muscles placed in saline active contractions. This was much more apparent in muscles that were partially detached, and especially noticeable in the sartorius when torn from the rest of the muscles and attached only by its lower end. Now these contractions must consume the energy of the muscles and hasten exhaustion and the advent of rigor mortis.

In February I commenced a further series of experiments regarding the effect of saline solution and saline solution containing other salts to produce contractions.

1. For permission to make these copious extracts, and to reproduce the accompanying traces, the thanks of the writer are due to Prof. J. N. Langley, the Editor of the *Journal of Physiology*.

Since I made these observations I find that Biedermann[1] has made similar experiments with results quite in accord with mine. Our methods however differed in some respects, and I recount here certain additional experiments to those he performed.

In his experiments he employed the sartorius of a strongly curarized frog. He suspended the muscle in a solution consisting of a litre of water, 5 grams NaCl, 2·5 grams Na_2HPO_4, and 0·4 gram Na_2CO_3, and in most of his experiments he kept the temperature below 10° C. He obtained contractions in all respects like those described in my experiments, but I employed all the muscles of the thigh cut into thin ribands and attached only at the knee. I did not curarize the frog and I placed the limb in simple saline. Biedermann took no tracings of the contractions.

It is obvious therefore that the alkaline soda salts do not play so important a part in the production of these contractions as Biedermann supposes. I shall show that phosphate of soda or caustic soda added to saline increases somewhat the frequency and the strength of the contractions. As I have said though working under different circumstances I obtain the same results, so well described by Biedermann.

We both find that temperature strongly influences these contractions, these being more frequent and more vigorous at a temperature between 20 and 30° C. than below 10° C. At a low temperature the contractions become somewhat rhythmical. There occur long pauses free from contractions, varying from ten seconds to two or three minutes, and then a series of contractions take place of much the same strength and separated by about the same intervals. Occasionally the beats for a minute or two are quite rhythmical.

I cut off both hind limbs of a frog through the hip joint and after stripping off the skin I cut the thigh muscles into thin ribands, and left them attached only at their lower end close to the knee joint. One limb I suspended in 200 c.c. ·6 per cent. saline solution, the other in a similar quantity of saline solution containing some other salt.

On placing the limb in saline solution the strips of muscles at once contract frequently, and one sees both massive and fibrillary contraction, i.e., the whole muscle or piece of muscle is contracted, and in addition one sees the contraction of fibrillae merely. The contractions begin soonest and are most marked in the thigh muscles cut into thin bands. Later fibrillary contractions occur in the uninjured lower leg muscles causing

1. *Wien. Sitzungsbericht.* Bd. LXXXII, 1880, p. 257.

twitching of the toes. These contractions persist often for hours and continue longer in the uninjured than in the thigh muscles cut into ribands.

As Biedermann noticed with his solution so I find that when the contractions have ceased they often recur on renewing the saline solution.

On removing the limb from saline, strong fibrillary contractions persist for some time, and when they cease the excitation of a contraction by the faradic current brings them back again to persist without any further stimulation for half a minute or even longer.

The muscles of the fellow limb placed in phosphate of lime saline solution twitch much less, and in some cases no contractions at all occur.

The addition of 1 c.c. of 1 per cent. solution of potassium chloride to each 100 c.c. of the testing fluid very slightly lessens the fibrillary twitching of the muscles in saline solution. The twitchings of the muscles in phosphate of lime saline solution are greatly lessened or even utterly arrested; but on replacing the muscles in their original fluid the twitching again increases, and on the further addition of potassium chloride the same effect follows as on the first addition.'

'I find that small doses of potassium chloride 1 c.c. to 4 c.c. of 1 per cent. solution added to 100 c.c. of saline somewhat lessen the contractions. These quantities added to phosphate of lime saline solution at once check any movements, though, as I have said, phosphate of lime without the aid of potassium chloride generally prevents any movements.'

'Saline made with tap water prevents these twitchings, as the following experiment proves.

On Jan. 20 I placed one posterior extremity in 200 c.c. distilled water saline, the other in 200 c.c. tap water saline. The muscles in the distilled water [saline] began to twitch in three minutes and in ten minutes became quite brisk, the lower leg muscles quivering actively as well as the partially detached muscles of the thigh. The twitchings continued all day and were quite energetic eight hours from the beginning. The muscles in tap water saline did not twitch at all.

Contrary to what one would expect distilled water causes few or no twitchings.

On Jan. 20 I placed one hind limb of a frog in 200 c.c. distilled water saline and the other in distilled water. Active twitching persisted in the muscles in saline solution three hours, but had ceased in five hours and a half. In the muscles in distilled water one twitch

occurred five minutes after the immersion but no other movement, and all the muscles soon passed into firm rigor.'

'Mere alteration in the percentage amount of salt influences the contraction, as the following experiment shows. On April 13 I placed the sartorius in 0·5 per cent. saline solution. For the first three minutes only the faintest contractions occurred. They then for two or three minutes became moderately strong and again grew weak. I then added 25 c.c. 2 per cent. solution of salt to 200 c.c. of 0·5 per cent., which much increased the activity of the contractions. I then returned to 0·5 per cent. solution and in about a minute the contractions became much weaker. I then added 25 c.c. of 2 per cent. solution to 150 c.c. of 0·5 per cent. solution and the contractions became greatly increased. I added another 25 c.c. of 2 per cent. solution and still further increased the contractions. Again I returned to 150 c.c. of 0·5 per cent. solution and the contractions greatly lessened, occurring in paroxysms with quiescent periods of half a minute. I then added 50 c.c. 2 per cent. solution, and again greatly increased the frequency of the contractions. On returning to 150 c.c. of 0·5 per cent. solution contractions ceased but returned for a short time on adding 50 c.c. of 2 per cent. solution of sodium chloride.

On the other hand, lessening the relative amount of NaCl, as by adding distilled water to the saline solution lessens or prevents these muscular contractions. For instance, reducing the 0·6 per cent. to 0·5 per cent. NaCl decidedly lessens the contractions. Here I will record one experiment showing the effect of dilution. I first took a trace with 200 c.c. 0·6 per cent. saline solution, and obtained active contractions. On adding 50 c.c. distilled water, the contractions ceased and failed to return on adding another 50 c.c. and a third 50 c.c. distilled water. On returning to 0·6 per cent. saline, slight contractions recommenced, and the further addition of 2 c.c. of 2 per cent. $BaCl_2$ induced active contractions.

I next tested the effect simply of distilled water on the contractions. I first took a trace with 0·6 per cent. saline solution, and then replaced this by distilled water, when the contractions ceased at once, though in some experiments a few single strong contractions occurred at many minutes' interval. The distilled water however induced some persistent contraction, which passed away on the addition of saline, provided not too long a term had elapsed, before adding the NaCl, i.e., about eight to ten minutes.

When these contractions brought out by immersion in saline cease

contractility still persists, and stimulating the muscle with a faradic current the coil, standing at 15 to 20, will excite good contractions. Moreover, the addition of substances like barium chloride at once developes active contraction; indeed barium chloride will produce contractions and persistent spasms much more marked than with saline at the beginning of the experiment.'

'I made twelve experiments to test also the effect of calcium chloride using 1 per cent. solution. I added 2 to 4 c.c. of this to 200 c.c. of saline solution. After testing the effect of simple saline I added at once 4 c.c. of calcium chloride solution. In every instance I tried this solution it almost immediately to a great extent lessened the contractions, and in many instances all but arrested them, in some, quite stopped them. In several cases after a few minutes slight contractions returned but were much less than with saline at the beginning of the experiment. In eight of the experiments at one period after the addition of lime chloride the contraction became literally rhythmic, i.e., the contraction occurred at pretty equal intervals and in much the same force. The following short account of an experiment is a good example of the experiments. On April 5 the sartorius in saline contracted actively. The addition of 4 c.c. of $CaCl_2$ solution at once almost arrested the contractions which were rhythmic in character. I then returned to saline solution, which induced active contractions, and these were at once arrested on the addition of 4 c.c. of calcium chloride solution. Again I placed the muscle in simple saline and again active contractions occurred, which were at once arrested on adding 4 c.c. of 1 per cent. solution of potassium chloride.

The addition of lime chloride arrests not only the rapid contractions but also removes the persistent contraction, so that the trace rises higher above the base line. These effects are shown in fig. 3.

In many experiments made with potassium chloride I find its action is largely influenced by temperature. My experiments have been made generally with the fluid at from 10° to 16° C. Soon after adding 4 c.c. of 1 per cent. solution of KCl to 200 c.c. saline solution the muscle strongly contracts, dragging down the recording lever far below the level reached even when the contractions are well marked with saline; the muscle then slowly relaxes, but before it has much elongated fresh contractions set in, or when at its maximum contraction frequent well marked contractions occur, and these cease and the muscle slowly relaxes, see fig. 4. Then the muscle remains quiescent for a variable

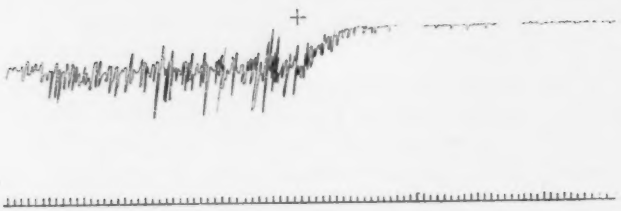


Fig 3

'Shows the effect of saline. At the cross 4 c.c. 1 per cent. $CaCl_2$ were added to 200 c.c. saline.'

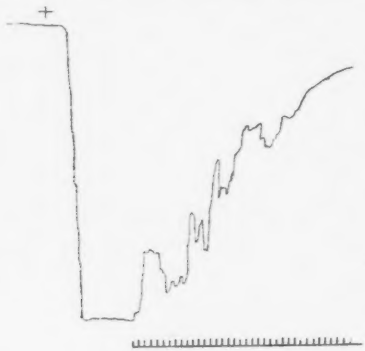


Fig 4

'Effect of adding 4 c.c. 1 per cent. solution of KCl to 200 c.c. saline solution added at the point indicated by a cross.'

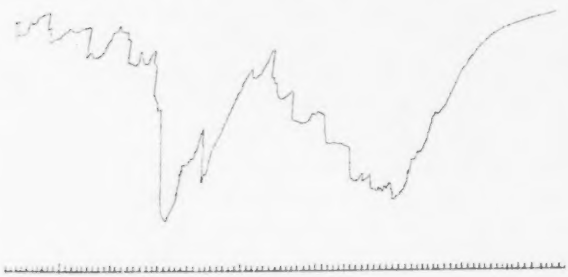


Fig 5

'Later effect of potassium chloride.'

time, ten seconds to one or two minutes, when contractions like those recorded in fig. 5 occur and again the muscle becomes quiet, or after a few minutes again contracts as shown in the figure. By degrees the contractions become less marked and at last merge into a single slight contraction which relaxes slowly. With some muscles only these slighter contractions occur throughout the experiment. With a higher temperature the addition of potassium chloride solution, just like calcium chloride at once arrests the contractions. 1 c.c. of 1 per cent. solution diminishes the contractions greatly, but 2 c.c., i.e., 1 part of KCl in 5000 well-nigh stops them or stops them altogether, and on returning to simple saline solution the contractions recommence actively.

The following experiment is a good example of the influence of potassium chloride. On Feb. 26 a sartorius muscle placed in saline contracted energetically. To the 200 c.c. of saline I then added 2 c.c. of 1 per cent. solution of potassium chloride and the contractions all but ceased. Again I placed the muscle in 200 c.c. of saline solution and active contractions returned. I again added 2 c.c. of potassium chloride solution and again greatly restrained the contractions, yet a third time saline solution restored the contractions, and this final trial stopped the contracting. In the previous experiment the changes were made quickly, but after the addition of 4 c.c. of potassium chloride solution to 200 c.c. saline solution, if the effect is watched for a considerable time, it generally happens that after an interval of two or three minutes contractions return, but occur in groups, a long interval often intervening. The contractions are often very vigorous and rapidly repeated; these outbursts of contractions at long intervals grow weaker and then cease. In some cases the trace is in the form of an irregularly wavy line.

Potassium chloride then whilst modifying considerably the character of the contraction induces very marked contractions, and our previous experiments showed that potassium chloride added to saline solution does not sustain contractility. The difference between calcium chloride and potassium chloride is very striking, as the following experiment shows. On May 6 saline solution excited active contractions, which were at once cut short on adding 4 c.c. of 1 per cent. solution of calcium chloride to 200 c.c. saline. After six minutes I replaced the sartorius in saline and soon after added 4 c.c. of 1 per cent. solution of potassium chloride. This produced the effect shown in fig. 5. I added 4 c.c. $CaCl_2$ solution and at once stopped all contractions. In about four minutes I replaced the muscle in saline without inducing any strong contractions, as generally

happens when saline solution replaces saline solution containing potassium chloride 1 part in 5000. Soon after I added 4 c.c. potassium chloride solution and again induced the usual potash effect, which was arrested again though more slowly by adding 4 c.c. of calcium chloride solution. I then again reverted to simple saline without inducing any contractions and again added 4 c.c. potassium chloride solution and induced well-marked potash contraction. These were again stopped though not so rapidly as on the first occasion by adding 4 c.c. of calcium chloride solution. Again I returned to saline, then added potassium chloride solution and again excited well-marked potash contractions, which calcium chloride once more arrested. This action of potassium chloride and the antagonism of calcium chloride I many times verified.'

In the later paper with Buxton it is shown that perfusion of the skeletal muscles with ordinary saline leads to similar contractions to which calcium salt acts again as a sedative. Skeletal muscle was found to differ from cardiac muscle in that, without any addition of calcium salt to the perfusing fluid, the period of relaxation became enormously prolonged, so that the trace became like that obtained from a veratrized muscle. Also, it was found that perfusion with sodium chloride saline alone very rapidly rendered the muscle inexcitable to direct faradization, but that excitability was restored on adding a calcium salt to the perfusing fluid.

Ringer's discoveries on the action of calcium salts upon contractile tissues and living organic processes in general led him to take a great interest in the studies of Hammarsten and J. R. Green on the action of the calcium salts in the coagulation of blood and milk, and to confirm and extend such observations in several important aspects. He showed that this favourable action of calcium salts was a generic property shared in feebler measure by the other members of the calcium group, and that it was to a certain extent antagonised by sodium and potassium, although this antagonism is but a feeble thing compared to that existing in living tissues. He also investigated the action of calcium salts upon metacasein and alkali albumin, showing that, following in exaggerated measure the general rule for inorganic calcium salts, the protein calcium salts are much less soluble at higher temperatures. These papers on the action of calcium upon coagulation contain much interesting material, and many valuable suggestions; they are contained in the *Journal of Physiology*, Vols. XI-XIII.

In the years 1894 and 1895, Ringer, in collaboration first with

Sainsbury,[1] and later with Phear,[2] studied the action upon *Tubifex rivulorum*, and upon growing tadpoles, of various saline constituents, and although the results contained in these papers, in so far as the supposed disintegrative action of distilled water is concerned, were vitiated, as demonstrated soon after by F. S. Locke,[3] by the presence of unsuspected minute traces of copper, yet the experiments on mutual antagonisms contained therein are still valid and valuable, as demonstrated later by Ringer himself (see footnote, p. iii).

In addition to the pioneer work which chiefly forms the subject matter of this sketch of Ringer's scientific labours, he published a text-book of therapeutics which ran through many editions, and from its originality of treatment denoted a scientific aspect of mind towards medicine somewhat rare at that time. His contributions to pathology and medicine are chiefly to be found in the pages of the *Practitioner*, as also more popular expositions of his bio-chemical researches. A biographical notice of Ringer's career, and of his work as a clinician, will be found in the *British Medical Journal*, October 29th, 1910, p. 1384.

BENJAMIN MOORE.

DECEMBER, 1910.

1. *Journ. of Physiology*, Vol. XVI, p. 1, 1894.
2. *Ibid.*, Vol. XVII, p. 423, 1895.
3. *Ibid.*, Vol XVIII, p. 319, 1895.

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CHRISTIAN A. HERTER

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IN MEMORY OF CHRISTIAN A. HERTER

[1865-1910]

A SKETCH OF THE LIFE OF ONE WHO CHAMPIONED THE CAUSE OF IMAGINATION AND IDEALISM IN THE MEDICAL SCIENCES

It is with deep regret that we chronicle the death of Christian A. Herter in New York on December 5th, 1910. The science of bio-chemistry and those branches of medicine upon which chemical research has thrown light have lost one of their foremost workers. Those who have had the pleasure of following his work closely must feel that the inspiration which permeated all that he wrote will be as much missed as his valuable contributions to scientific knowledge. Though his death occurred at the comparatively early age of forty-five, he has left an indelible mark on the medical science of his time. His personal qualities, wide influence, and inspiration, will be greatly missed. While perhaps he is best known in this country for his scientific work, there was a wider side to his character which permeated the medical science of his country, and in no small degree reached his readers on this side of the ocean. In his last public utterance he put his spirit into words, by which his life and work may well be judged. In an address on 'Imagination and Idealism in the Medical Sciences,'* he said: 'I like to think of medicine in our day as an ever-broadening and deepening river, fed by the limpid streams of pure science. The river at its borders has its eddies and currents, expressive of certain doubts and errors that fringe all progress; but it makes continuous advances on the way to the ocean of its destiny.'

Garnering from the fertile field of history, in this address he briefly and lucidly depicts the motives underlying the work of some of the Masters of Medicine, showing 'that the history of medical discovery is a long chain of imaginative experiences whose links have been welded and fixed by passing through the fiery ordeal of appeal to experimental tests.' . . . . 'There is a phase of imaginative thought and feeling which expresses itself in a strong desire to pursue ideal ends, even at the cost of the ordinary prizes of life, wealth, material power and physical comfort. This idealism has been a very pronounced attribute of the great masters of medicine.'

* Delivered at the opening of the Medical School of the College of Physicians and Surgeons, Columbia University, in September, 1909.

Apart from Herter's contributions to science, workers, not only in America, but elsewhere, have reason to believe that in the realisation of these ideals in his own life, medicine has gained a great deal. Convinced, as he was, that medicine was destined to become based on exact science, he in his early days, when in the actual practice of his profession, established a laboratory in his own house for the investigation of problems closely connected with his daily work. Later, this spirit needed little appeal to invoke his aid for the development of the Rockefeller Institute for Medical Research. The conception of medical work initiated on a small scale in his early days has now its full opportunity in that Institution. Ever with his mind on the direct and present value of research to medicine, he was convinced of the need of a hospital as an integral part of the scheme. This institution has already done so much, not only for science, but directly for the alleviation of human suffering, that its founders have early reaped a rich reward. It is sad that Herter was not destined to see further results of his labours in this direction.

In his comparatively short life, Herter has left his mark on many fields of activity. He graduated at the College of Physicians and Surgeons, Columbia University, in 1886. After a period of post-graduate work under Professor Welch at Johns Hopkins University, he studied with Professor Forel in Zurich, Switzerland. In 1892 he published a work on 'The Diagnosis of Diseases of the Nervous System.' At this time he was in medical practice, mainly of a neurological character. Soon the field of medical chemistry attracted him, and he relinquished practice. The result of his having actually practised his profession is to be traced throughout the whole of his later work. In the majority of his papers the therapeutical bearing of his work is considered. In 1897 he was appointed Professor of Pathological Chemistry in the University of Bellevue Hospital Medical College. The lectures delivered while occupying this chair were published in 1902. The pleasure which we had in reading this work will not be forgotten, and we can well believe that it has been an incentive to many, as to the writer, to enter the field of research in the chemical problems of disease. On many occasions we have heard young graduates express their keen appreciation of the instruction and inspiration they have found in its pages. As in all his writings, the points are made directly and clearly. The chapter, in particular, on the chemical defences of the organism against disease, shows that he was peculiarly gifted in being able to express deep problems in the simplest and most expressive language. He was called

to the Chair of Pharmacology and Therapeutics in the College of Physicians and Surgeons, Columbia University, in 1903, and occupied that position up to the time of his death. The wisdom of appointing a chemist to the Chair in this subject is demonstrated by the valuable light Herter has thrown on the diseases of the gastro-intestinal tract, and their treatment. His last work on Infantilism shows how his researches yielded valuable results to the clinician, not only in the recognition of morbid conditions, but in modes of attack on diseases for which hitherto little could be done.

Though the major portion of his time was spent in research, he kept closely in touch with medicine. For ten years (1894-1904) he was visiting physician to the New York City Hospital on Blackwell's Island. His interest in and aid to the hospital of the Rockefeller Institute naturally led to his being appointed Physician to the Hospital in 1908.

His interest in medicine and ancillary sciences was as broad as it was active. He founded the *Journal of Biological Chemistry*, and with Professor Abel was one of its first editors. The scope of the journal was to include researches of a purely scientific character, but, having in mind the needs of clinical medicine, an effort was made to encourage contributions to the knowledge of the chemical aspects of disease. He ensured the permanent stability of the journal, provided a Board of Directors for the perpetuation of the publication, and incorporated it under the laws of the State of New York.

Imbued with the idea of the diffusion of medical knowledge, he founded two lectureships, one in Baltimore, the other in New York. By these lectureships distinguished European scientists are invited to deliver lectures in America. The Baltimore lectures have been given by Ehrlich, Han Meyers, Schaefer, and Chiavi. To this list, but for his untimely death, would have been added the name of Weigert. The New York lectures have been delivered by Halliburton, Levene, von Noorden, Friederick Müller, Starling, Leathes, and Cohnheim. He was active in the foundation of the Harvey Society of New York.

Before reviewing his scientific work, it is of interest to note the source of the idealism in medicine which he practised as well as preached. His addresses are full of a ripe knowledge of the history of medicine, and we can conceive that it was from this source that he himself obtained some of the inspiration with which his life was filled, and which he handed on so liberally to others. In an address[1] delivered before the Medical School of Johns Hopkins University, in 1904, he chose as his

subject 'The Influence of Pasteur on Medical Science.' He considers that master as a person, an investigator, and as a public benefactor, thus reflecting those traits which made himself a man whose loss is felt.

In this address we see the association of science and medicine which is to be found in all his writings. The address emphasises the value of a closer association between the two. 'In looking for fresh proofs of bacterial origin of disease, Pasteur made some visits to the hospitals of Paris, and thus came into closer relations with the practice of medicine and surgery. The alert and intellectually honest minds bade him welcome, and gave him every help to pursue his studies; the conservatives looked at him askance, confidently set up their time-worn theories against his experimental proofs, and lost no occasion to ridicule the germ theory of the origin of disease.'

Herter was convinced of the value of chemistry and physics in the armamentarium of medicine, as the following glimpse of Pasteur in the same address shows:—

'Perhaps the most deeply significant feature of Pasteur's contributions to medicine is their direct dependence on the principles of physics and chemistry, the sciences that so often lie at the heart of real advances in biology. Medical men trained along the conventional semi-scholastic lines had often dabbled with these fundamental sciences, and sometimes the superficial contact had yielded creditable or even important results. In many instances, also, truly great advances had come from the labours of men who, like Malpighi, Bichat, and Johannes Müller, were wide awake to the fact that sound medicine must rest on sound biological conceptions. But despite the activity of numerous medical men of broad scientific sympathies, the medical profession at the beginning of Pasteur's career was dully following a well trodden but nearly blind road, in the hopeless struggle to solve the intricate problems of human pathology and physiology by minute observations and experiments confined largely to the most complex representatives of animal life. Then for the first time there appeared in the biological sciences a man profoundly trained in the methods of chemistry and physics, and inspired, moreover, with a firm confidence in the applicability of these sciences to the solution of biological and medical problems. Triply armed with a sound method, a lofty imagination, and a strong will to serve his country, Louis Pasteur entered the wide arena of medical research, to win there the triumphs that have reconstituted medicine, and have secured him an undying fame.'

Herter's contributions to medical science show the imprint of those ideals. He approached the problems of disease in a broad spirit, with a wide knowledge of those sciences above mentioned. He confined himself to no narrow view, but approached his problems from all sides. Bacteriology, chemistry, clinical manifestations, and even sociological aspects, all contributed their quota to his studies of disease. In a review of the collected works of Carl Weigert, written in collaboration with E. K. Dunham, Weigert is placed in a select group of deliberate careful workers, who regard a problem calmly and from every side, while bringing to bear extraordinary powers of analysis and a high degree of ingenuity in overcoming technical difficulties. Weigert's mind was one that advanced step by step, and took few risks. This description holds good for Herter's own work. Throughout the whole of his researches on the bacterial infections of the intestinal tract, we see those characteristics.

The Works of Herter

Herter's greatest work was the one just mentioned, his contributions to the study of the complex elusive diseases set up by the growth of an abnormal flora in the intestinal tract. He fully demonstrated that certain species of organisms in the intestines produced abnormal chemical products which were absorbed, and that these conditions were associated with chronic forms of disease. Nearly all of his contributions of later years deal with this subject. Methods of separating, identifying and studying the complex flora were evolved in these researches, which are now indispensable to the workers in the subject. The effects of the various organisms on the different physiological principles of food are considered, and particular attention is given to the disruptive products of the proteins, or their combinations, which appear in the urine in abnormal conditions. The end always in view is the association of the abnormal flora, and the toxic bodies produced, with the form of disturbance manifested clinically.

The real significance of the intestinal flora is considered to lie, not in any immediate relation to processes of digestion, but in a wholly different direction. It is impossible to avoid the entrance of bacteria into the digestive tract. The obligate bacteria (for example, *B. lactis aerogenes*, *B. coli*, *B. bifidus*) adapt themselves to the secretion of this part of the body, and ordinarily hold their own against new comers. By

virtue of their adaptation, they are not ordinarily harmful to their host, but, on the contrary, they are, under some circumstances, capable of doing service by giving rise to conditions that discourage growth of many harmless and harmful species which man cannot readily exclude from his digestive tract.[2]

'A long, largely anaërobic intestinal tract permitting gradual resorption of the contents is a physiological necessity in order that a loss of water and its detrimental consequences may be spared the organism. The presence in the colon of immense numbers of obligate micro-organisms of the *B. coli* type may be an important defence of the organism in the sense that they hinder the development of that putrefactive decomposition which, if prolonged, is so injurious to the organism as a whole. This adaptation is the most rational explanation of the myriads of colon bacilli that inhabit the large intestine. This view is not inconsistent with the conception that under some conditions the colon bacilli multiply to such an extent as to prove harmful through the part they play in promoting fermentation and putrefaction.'[2]

In his work he uses the term fermentation to designate the decompositions of carbohydrate and fatty substances, and the word putrefaction he applies to the cleavages of protein and allied substances.

The non-motile form of organisms like *B. lactis aerogenes*, *B. acidophilus*, staphylococci, and streptococci, enter the sterile digestive tract of the new-born by the mouth, the members of the colon bacillus group by the mouth or the anus, but the *B. bifidus*, the chief obligate bacterium of the milk stools of nurslings, reaches the intestinal tract only by the anus.

His work shows how by careful employment of Gram's stain a rough differentiation of bacterial types in the intestine can be obtained, as the positive and negative reactions correspond to bio-chemical peculiarities of the bacteria. The faeces of the nursling child thus stained present characteristic appearances. The bacteria are extremely abundant—more so than in the adult—and a large majority of them are Gram positive. They may give the field the appearance of consisting of a nearly pure culture of one bacterial type.

By supplementing this method of study by making aërobic and anaërobic cultures on various media these were found to be *B. bifidus*. Very like these, and found in the faeces, was *B. acidophilus*. Other Gram positive organisms found, though few in number, were *B. aerogenes capsulatus*, and a diplococcus, which frequently grew in

chains. The chief Gram negative bacilli are small cocci, and shorter and longer bacilli which by cultural methods can be shown to have the biological characters of the *B. lactis aerogenes* and *B. coli communis* group. By a comparison of microscopic fields with cultural methods a knowledge of the distribution of the bacterial flora was obtained.

In bottle-fed children there are some important differences. The place of importance is in their case occupied by organisms of the *B. coli* type, and the majority of organisms are Gram negative instead of Gram positive, as is the case in nurslings.

After infancy the more varied diet increases the opportunities for the entering of many kinds of bacteria into the digestive tract, and although individual variations are considerable, Herter describes conditions that are fairly typical for persons in good health and favourable environments. During childhood and adolescence there is a slow transition from the conditions of infancy to those of adult life; *B. bifidus*, though present, is much less numerous, and other types are more numerous. Still the numbers of putrefactive anaërobes are small, and putrefaction processes in the intestine are not active. Toward adult life great differences exist in the habits of different persons, and these are in a degree reflected in the nature of the bacterial processes of the digestive tract. Herter succeeded, by elaborate and painstaking research, in bringing some order into the apparent chaos of these differences. Further, he succeeded in demonstrating the important fact that varying types of flora could be associated with definite manifestations of disease. A mere summary of his findings can give but little idea of the vast amount of careful work done to elucidate these difficult and apparently insurmountable problems. Various animals were observed, and in a contribution in collaboration with A. I. Kendall it was shown that the same bacterial changes occur with the same types of diet in the different animals, irrespective of any novelty of the food supplied to the animal under observation. There is an antagonism between the flora generated by a protein and a carbohydrate diet. Changes from one diet to the other are regularly accompanied by a corresponding change in flora.[4] These changes are accompanied by alterations of the cleavage products in the intestine which are absorbed and then excreted by the urine. Associated with these is a definite change in the clinical conditions of the animal. With the advance of age the condition of well-being is closely related to the absence of putrefactive anaërobes from the intestinal tract. The onset of senility may be distinctly accelerated through the development of intestinal

infections in which the putrefactive anaërobes are prominently represented.[2]

In the elaborate and careful methods always used the morphological and cultural characters of the organisms were thoroughly considered, but, in addition, considerable attention was paid to their bio-chemical properties. Much valuable information was obtained from a study of the cleavage products produced by the action of organisms on the soluble carbohydrates. The study of the anaërobic life of the large intestine in health and disease, based upon these methods, is a masterpiece of careful research.

In the course of his researches, new problems arose and old ones were reconsidered. The effects of cleavage products produced, on the urine, and the reaction for these bodies or their compounds, afforded some extremely valuable contributions,[5] as did also the bio-chemical activities of the organisms themselves.[6]

Valuable as these contributions are, the particular genius of Herter has made them of the greatest possible utility to medicine by their co-ordination with a close study of the associated manifestations in the organism. 'The considerable variations in the clinical manifestations and pathological accompaniments of chronic intestinal putrefaction suggest that the aetiological conditions vary considerably in different cases. I shall endeavour to show that there are grounds for the separation of different types of intestinal putrefaction processes, and that these grounds are based on differences in the character of the putrefaction products of the bacteria concerned with these processes. The proposed classification recognises three types of putrefaction which are common.'

These types of intestinal disorder are:—

The INDOLIC, which is characterised by indicanuria dependent chiefly upon excessive decomposition inducted through the co-operation of members of the *B. coli* group (and probably *B. putrificus*).

The SACCHARO-BUTYRIC, which is initiated especially by the anaërobic organisms in the digestive tract. In its simplest form it is associated with little production of indol in the intestinal tract, and hence with slight indicanuria, or even an entire absence of indican from the urine.

The *combined type* of indolic and saccharo-butyric types of decomposition.

The numerous products produced by these fermentations and

putrefactions were investigated in detail, the basic substances, sulphur compounds, and aromatic products of the first type,[2] and the gas and fatty acids of the second type. Especially noteworthy is the work on indicanuria[2] and indolaceturia.[7]

The formation of indol is ascribed to an unusual activity on the part of the colon bacillus. It occurs in cases of complete obstruction of the bile duct, in cases of functional pancreatic achylia and occlusion of the small intestine. Mere constipation does not necessarily lead to it. A large group of cases occurs in the marantic large belly type of chronic intestinal indigestion in children, with retardation in growth.

The saccharo-butyric type of chronic intestinal disorder has its seat mainly in the large intestine and lower ileum. It is due to the action of very large numbers of strictly anaërobic butyric-acid-producing bacteria, capable of multiplying by spore formation. The organism most prominently concerned is the *B. aerogenes capsulatus*.

The faeces in this type contain little or no indol, and there is consequently little or no indicanuria. It is a widespread condition among adults, and is relatively uncommon in children. It sometimes happens that people may have this condition for a long time without any distinct impairment. On the other hand, a condition of distinct invalidism may occur, and life be considerably shortened in consequence. There is a tendency to desquamation of the epithelium of the digestive tract, often marked in the mouth and on the tongue. This epithelium is to be found in the stools which have a tendency to be diarrhoeic. The condition may be temporary or permanent. The organism is capable of producing haemolytic substances, and in many of the cases there is a distinct anaemia. In an important contribution[8] instances are given of primary 'pernicious' anaemia, and others showing the blood changes of secondary anaemia (of undeterminable aetiology) in which there is chronic infection of the intestinal tract with *B. aerogenes capsulatus*. The living *B. coli* are much reduced in these cases.

In the combined form nervous symptoms are apt to be prominent early in the affection, and a state of chronic invalidism is reached more rapidly than where either condition alone is prominent. Almost every known expression of functional disorder of the nervous system was noted in the subjects of this variety of intestinal putrefaction.

Continuing the researches to their logical conclusion, methods of treating these conditions were devised and studied. These relate to the avoidance of bacterial contamination of the food, to the promotion of

prompt digestion in and absorbtion from the small intestine, and finally to agencies designed to reduce the numbers of putrefactive anaërobes living in the intestinal tract.

His last work,[3] a study of the clinical course, bacteriology, chemistry, and therapeutics of arrested development in infancy, shows the value of the methods which he had built up for the study of intestinal disorders. Bramwell's work shows the rarity of arrested growth in children due to disordered secretion. Herter has supplied a long-felt want in contributing something practical to this subject. The essential clinical features of the cases he describes are (1) an arrest in the development of the body, (2) the maintenance of mental powers and fair development of the brain, (3) marked abdominal distension, (4) moderate grades of anaemia, (5) rapid onset of physical and mental fatigue, (6) various obtrusive irregularities referable to the intestinal tract.

These cases are characterised by the presence of a dominant Gram positive flora in the digestive tract, and the central aim in the treatment laid down is the modification of this flora.

A review of Herter's work at once suggests the fields of research which it will open to future workers. His was the plough in virgin soil, and the fruits of his labours will be reaped in many directions. His life and work can be well expressed in the words of one of his colleagues.* 'The physician's life should be one of service and leadership combined. He serves well when he relieves suffering; still better, when he teaches men how to live; but he serves best of all when he pushes out into the unknown and makes medical science the richer for what he contributes to it. The knowledge of wise men, the deeds of diligent men, and the valour of heroes are the gifts of those who have preceded him. Let us see to it that we pass on this heritage, augmented, to those who follow.'

OWEN T. WILLIAMS.

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